Assessment of Health Risks from Exposure to Acrylamide

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Office of Toxic Substances
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PREFACE

The present document includes the original risk assessment of acrylamide which was completed in March 1988, plus the following revised or additional sections, completed after March 1988:

Section 8.2 - Epidemiology Studies. This section has been revised to include evaluation of data on a third epidemiological cancer study in humans.

Section 8.4 - Second Lifetime Oncogenicity Study in Rats with Acrylamide. This section evaluates the bioassay study conducted by American Cyanamid Company regarding the carcinogenicity of acrylamide. A Final Report was completed by American Cyanamid on June 27, 1989.

Section 12 - Summary of Public Comments and EPA Responses on Preliminary Assessment of Health Risks from Exposure to Acrylamide. Comments were received in response to a Federal Register notice of February 28, 1989 (Vol. 54, No. 38) requesting comments on 13 chemicals proposed for addition to the National Toxicology Program Sixth Annual Report on Carcinogens and other proposed actions relevant to that report. This section summarizes the public comments received plus the EPA responses. Additionally, several arithmetic errors noted in the public comments have been corrected in the appropriate tables..

In addition, the OSHA PEL for acrylamide has been revised according to the Federal Register of January 19, 1989. The new limit has been substituted and is used in the present text.

The number of exposed persons engaged in sewer grouting has also been updated.

1. EXECUTIVE SUMMARY

1.1. Hazard Assessment

The available data provided a basis for identifying several human health hazards related to acrylamide exposure. The identified hazards include: neurotoxicity, carcinogenicity, genotoxicity, reproductive effects, and developmental effects. The available evidence for these hazards is sufficient to conduct quantitative risk assessments for neurotoxicity, carcinogenicity, reproductive effects, and to a limited extent for genotoxicity, but is not sufficient for such purposes for developmental effects.

Neurotoxicity from acrylamide exposure has been observed in both humans and laboratory animals. Neurotoxic effects in both the peripheral and central nervous systems in humans have been observed, including irreversibility of effects in some human case reports. The best animal data indicate no observed effect levels (NOELs) and lowest observed effect levels (LOELs) of 15 and 25 mg/kg, respectively, following a single dose, and 0.2 and 1.0 mg/kg/day, respectively, following chronic exposures.

Based on the available data on carcinogenicity, there is evidence according to EPA's Cancer Risk Assessment Guidelines to identify acrylamide as a "B2-probable human carcinogen." This finding is based on the following: occurrence of benign and malignant tumors, tumors observed in males and females, tumors observed at multiple sites, tumors observed in 2 animal species (one tested in a lifetime bioassay and one tested in a limited

bioassay), and a general dose-response observed in a lifetime bioassay. Also, the genotoxicity and metabolism data indicate that acrylamide can interact with and damage DNA material. Two epidemiologic studies are available, but they are "inadequate" according to EPA's Cancer Risk Assessment Guidelines for assessing acrylamide induced carcinogenicity.

The genotoxicity data on acrylamide provide a basis to support the carcinogenicity hazard identification and to support heritable mutations as a separate endpoint of concern for acrylamide exposures. The major concern for the genotoxicity of acrylamide is its clastogenic activity, which appears more pronounced in the germ cells than in the somatic cells based on *in vivo* assay results. The interaction with the germinal tissue suggests the possible heritability of acrylamide induced mutations in the human population. Under EPA's Guidelines for Mutagenicity Risk Assessment, only direct human evidence could increase the human germ-cell mutagenicity concern.

A reproductive hazard from acrylamide exposure has been identified based on the results of a growing body of animal studies. These studies demonstrated physical and biochemical reproductive changes in rats and mice following acrylamide exposures, including: sperm head abnormalities, dominant lethal effects, decreased mating capability, testicular atrophy, and changes in testosterone levels. Some of these effects may be related to acrylamide's genotoxicity and neurotoxicity, but since reproductive effects have been observed in 2 species in the

absence of other apparent systemic toxicity, the available data indicate that acrylamide has the potential to act directly on the reproductive system, rather than through stress or other systemic effects. These data suggest a NOEL and LOEL for reproductive effects of about 9 and 4 mg/kg/day, respectively.

Although there is some evidence that acrylamide may cause developmental effects, the basis for this conclusion is relatively weak because of the confounding of test results by acrylamide's neurotoxicity and questions about the toxicological significance of the biochemical changes seen. A LOEL of 20 mg/kg/day has been identified for this hazard (biochemical changes) from the available animal data. A NOEL cannot be identified from these data.

The available data provide a good general understanding of the metabolism of acrylamide (including: absorption, distribution, biotransformation, and elimination). However, there is still a lack of detailed pharmacokinetics for certain aspects of its metabolism such as for dermal absorption rates. Studies of the processes or mechanisms through which acrylamide produces its various toxic effects, especially its neurotoxicity, have begun to yield data on its mode of action. Binding to cytoskeletal elements and disturbance of enzymes involved in energy transfer have been hypothesized as mechanisms of action.

1.2. Exposure Assessment

Acrylamide monomer is produced by three companies at three sites in the United States. In 1985, production was approximately 140 x 10^6 pounds. Nearly all acrylamide produced is converted to various kinds of polymer products. Only about 5% is used in monomeric form. Some of this is finally converted to a polymer to perform the desired function, e.g., soil grouting and polyacrylamide gel electrophoresis.

Polyacrylamide products are used in potable water and wastewater treatment, oil well operations, mineral processing, sugar refining, and papermaking. All of these products may contain residual levels of acrylamide monomer, but the concentration can be expected to vary depending on the chemical structure of the polymer and the intended use.

The groups exposed to the highest levels of acrylamide are the acrylamide manufacturing/processing and sewer grouting sectors. Monitoring performed by NIOSH in 1985 indicates that persons engaged in the manufacture/processing of acrylamide are exposed to 8-hour time-weighted averages (TWA's) that range from 0.001 to 0.132 mg/m^3. Fewer than 200 persons are directly involved in the manufacture of acrylamide, while about 800 are involved in the conversion of acrylamide to polyacrylamide. The OSHA PEL is 0.03 mg/m^3 (skin). ACGIH reduced its recommended level from 0.3 to 0.03 mg/m^3 in response to the cancer data.

The 1800 persons engaged in sewer grouting may be subject to airborne levels of acrylamide that are at the low end of the

range experienced in manufacturing. However, dermal exposure may be high. Field monitoring conducted by EPA showed airborne levels that ranged from nondetectable to 0.12 mg/m^3, with dermal exposure levels ranging from 0.484 to 4.8 mg/hr.

Of the polyacrylamide exposure categories, consumers of potable water (4.5×10^6 to 30×10^6 persons) treated with polyacrylamide flocculants are potentially exposed to acrylamide levels of up to 0.5 ppb in the water, or 1.4×10^{-5} mg/kg/day. Other users of polyacrylamide products are believed to be exposed to relatively low levels given the use characteristics of the polymer products and the amount of residual acrylamide in the polymers.

One group that is potentially exposed to high, but varying, intermittent levels of acrylamide are the approximately 100,000-200,000 persons who use acrylamide to prepare gels for electrophoresis. Exposure levels are unknown, but the potential for significant dermal exposure exists.

1.3. Quantitative Risk Assessment

Risk estimates for each of the various exposed groups have been calculated for the risk of cancer, neurotoxic and reproductive effects, the latter two in terms of margins of exposure. These hazards were selected as the focus of risk estimation because: 1) carcinogenicity is modeled as a non-threshold effect; 2) neurotoxicity has been observed in humans and at low doses in a variety of animal studies; and 3)

reproductive effects have been observed independent of neurotoxicity or other systemic effects at the lowest doses for the identified threshold effects. While of high concern, genotoxicity risks (heritable mutations) were only preliminarily done because of limitations in the genotoxicity data base.

Persons involved in sewer grouting face 10^{-3} to 10^{-1} cancer risks as well as low or no margins of exposure (MOE) for neurotoxic and reproductive effects. Even accounting for less than 40 years of exposure, eight hours per day, etc., cancer risks are still in the 10^{-3} to 10^{-2} range (grouting does not occur daily or year-round). Neurotoxicity risks are more difficult to assess because of the intermittent nature of sewer grouting. Even so, the observation that one employee was observed with and two reported symptoms characteristic of peripheral neurotoxicity during the EPA field monitoring survey indicates the potential for significant dermal exposure and high risk. Such symptoms could be expected based on the monitoring results.

Manufacturing and processing employees face 10^{-3} to 10^{-2} cancer risks based on air monitoring data alone. Margins of exposure for neurotoxicity and reproductive effects range from about 15 to 300.

Consumers of drinking water face cancer risks of about 10^{-6} and are at no significant risk from neurotoxic and reproductive effects (MOE's of >> 1,000).

Because no exposure data are available for persons exposed to residual acrylamide in other polyacrylamide products,

quantitative risk estimates are not possible. However, given the low concentration of acrylamide in these products and the intermittent exposure patterns expected, risks may be low.

Finally, although the risk of heritable mutations cannot be readily quantified, the qualitative weight of evidence is high. Only human evidence could make the case stronger. Consequently, in the absence of information to the contrary, all exposed groups must be considered to be at some level of risk with the highest being grouting workers.

2. INTRODUCTION

Acrylamide is currently regulated by the Occupational Safety and Health Administration (OSHA) to a permissible exposure limit of 0.03 mg/m^3 (skin) as an 8-hour time-weighted average. The OSHA standard is based on acrylamide's neurotoxic properties.

On April 19, 1978 (43 FR 16684), the Interagency Testing Committee (ITC), established under section 4(e) of the Toxic Substances Control Act (TSCA), recommended that acrylamide be tested for carcinogenicity, mutagenicity, teratogenicity, and environmental effects; and that epidemiologic studies be performed. The ITC based its testing recommendations on high production (1976 production of 64 million pounds), anticipated 12% production growth rate over the following decade, exposure of an estimated 20,000 workers, possible widespread and low-level exposure of the general population due to its various uses, and insufficient testing information on the recommended effects.

As its response to the ITC, the Agency published (July 18, 1980; 45 FR 48510) its tentative decision not to require health effects testing (environmental effects will be separately assessed). This decision was based on the assumption that any controls set on the basis of acrylamide's neurotoxicity would likely provide a reasonable degree of protection from other potential health effects except cancer. Because the Dow Chemical Company, in concert with the American Cyanamid Company, Nalco Chemical Company, and Standard Oil Company (Ohio), had initiated

a cancer bioassay, it was not necessary to initiate additional oncogenicity testing. After reviewing the public comments in response to the 1980 notice, the Agency published its final decision not to require health effects testing (July 31, 1984; 49 FR 30592). By that time, positive preliminary results of the Dow bioassay had been submitted to EPA.

On December 15, 1982, Dow submitted a progress report on the bioassay study and reported an increased mortality for female rats at the highest dose level and an increased number of female rats with a grossly observed mass in the mammary region. Subsequent submissions on July 15, 1983 and March 14, 1984 reported statistically significant numbers of malignancies in female and male rats, respectively. As a consequence of the July 1983 report, EPA used an existing interagency agreement with the National Institute for Occupational Safety and Health (NIOSH) to conduct workplace air monitoring at acrylamide manufacturing and processing facilities and a soil grouting site. This monitoring occurred during 1984. Results were received in early 1985.

The final report of the Dow oncogenicity study was received in November 1984. Significant increases in tumors were reported for three sites in the male rat and at seven sites in females. As a result of these data, and the NIOSH monitoring results, additional monitoring was done at soil grouting sites because of the potential for significant dermal exposure. This monitoring was conducted in the Spring of 1986 and the results are a part of this risk assessment.

Because of the potential for very low levels of acrylamide in potable water from the use of polyacrylamide flocculants, which contain residual acrylamide monomer, the Agency proposed a Maximum Contaminant Level Goal (MCLG) in drinking water for acrylamide (November 13, 1985; 50 FR 46936). MCLG's are health goals. When the MCLG is promulgated, the Agency will propose a maximum contaminant level, which is an enforceable standard for acrylamide.

This risk assessment will thus serve as a source document for risk management decisions for a number of programs in EPA plus local and state governments and others.

3. PHYSICAL-CHEMICAL PROPERTIES

3.1. Physical and Chemical Properties of Acrylamide

3.1.1. Physical Properties

Acrylamide, $CH_2=CHCONH_2$, is the parent compound of a large group of monomers that includes methacrylamide, $CH_2=C(CH_3)CONH_2$, and numerous other N-substituted derivatives. Examples of these latter compounds are: N-isopropylacrylamide, N-tert-butylacrylamide, N-methylolacrylamide, and N,N′-methylenebisacrylamide (GCA, 1980).

Acrylamide, also known as 2-propeneamide or propenoic acid amide, is a white, crystalline solid with a very low vapor pressure (0.007 mmHg at room temperature). Pertinent properties for pure acrylamide are given in Table 3-1. Because most acrylamide today is produced and shipped in aqueous solution, the physical properties of 50 percent aqueous acrylamide are provided in Table 3-2.

Table 3-1. Chemical and Physical Properties of Acrylamide*

Property	Value
Molecular weight	71.08
Freezing point, °C	84.5 ± 0.3
Boiling point, °C at 2 torr	87
at 5 torr	103
at 25 torr	125
Density, g/cm^3 at 30°C	1.122
Refractive indices: n_x	1.460 (calculated)
n_y	1.550 ± 0.003
n_z	1.581 ± 0.003
Crystal system	Monoclinic or triclinic
Crystal habit	Thin tabular to lamellar
Ultraviolet spectra	End absorption starting at 280 nm log = 0.5 and increasing to log = 4 at 200 nm; no max in the near UV
Vapor pressure	
Liquid, torr at (°C)	2 (87)
	5 (103)
	10 (116.5)
	25 (125)
Solid, torr at (°C)	0.007 (25)
	0.033 (40)
	0.07 (50)
Heat of Polymerization (kcal/mole)	-19.8
	-20.4

Table 3-1. Chemical and Physical Properties of Acrylamide*

Continued

Property	Value
Solubilities (in g/100 ml at 30°C)	
Water	215.5
Methanol	155
Ethanol	86.2
Acetonitrile	39.6
Dioxane	30
Acetone	63.1
Chloroform	2.66
Benzene	0.346
Ethylacetate	12.6
n-Heptane	0.0068
1,2-Dichloroethane	1.50
Dimethylformamide	119
Dimethylsulfoxide	124
Pyridine	61.9
Carbon tetrachloride	0.038

Note: g/cm^3 = grams per cubic centimeter
nm = nanometers
UV = ultraviolet
kcal/mole = kilocalories per mole
g/ml = grams per milliliter

*Table taken from Environmental Science and Engineering (1981)

Table 3-2. Physical Properties of Aqueous Acrylamide*

Property	Value
Assay, (Wt % acrylamide)	48-52
pH	5.0-6.5
Polymer, max %	0.05
Refractive index range @ 35°oC (95°F)	1.4080-1.4146
Viscosity, cps @ 25°C (77°F)	2.71
Specific gravity, @ 25°C (77°F)	1.0412
Density, 25/4°C	1.038
Crystallization point	8-13°C (47-54°F)
Boiling point	99-104°C (210-220°F)
Vapor pressure kPa 23°C (mm Hg)	2.4 (18)
31.2°C (mm Hg)	4.0 (29.8)
37.8°C (mm Hg)	5.8 (43.4)
52.7°C (mm Hg)	12.4 (92.7)
70.5°C (mm Hg)	27.9 (209.5)
Specific heat (20-50°C, range)	0.83 cal/(g-deg)
Heat of dilution to 20 wt %	1.1 cal/g of solution (or 2.0 Btu/lb of solution [exothermic]
Heat of polymerization	20.4 kcal/g mole [exothermic]
Heat of melting (solution) melting range -17.3 to + 19.7°C	59.2 cal/g (106.5 Btu/lb)
Flammability	Nonflammable

*Table taken from CGA (1980).

3.1.2. Chemical Properties

Acrylamide is capable of undergoing numerous reactions at both the amide group and at the carbon double bond. Reactions of the amide group include hydrolysis to acrylic acid, dehydration to acrylonitrile, alcoholysis to acrylic esters, and condensation with aldehydes. Reaction with formaldehyde is commercially important and leads to N-methylolacrylamide, N,N'-methylenebisacrylamide, and N-isobutoxymethylacrylamide. Reactions at the double bond include addition to hydroxy compounds, amines, ammonia, mercaptans, bisulfite ion, Diels-Alder reactions, and polymerization (alone or with comonomers). Those reactions involving polymerization, preparation of N-methylolacrylamide, and bisulfite ion are among the most important commercially, and are briefly discussed below (GCA, 1980).

In the presence of free radicals, acrylamide polymerizes rapidly to polymers of molecular weight 200,000 to 10,000,000. Common initiators are peroxides, azo compounds, redox pairs, photochemical systems, and x-rays. In practice, redox couples such as sodium persulfate and sodium bisulfite are typically used. The highest molecular weights are obtained in water. Pure polyacrylamide made by free radical mechanisms is a colorless, odorless material, generally produced as both a liquid and a powder. In solid or bulk form, it is hard and glassy, softening at 188-210°C (370-410°F). The polymer is highly soluble in water and tolerates high levels of dissolved inorganic salts.

Copolymers with acrylamide can be easily prepared, although molecular weights are lower than those of polyacrylamide prepared similarly. Copolymerization by free-radical mechanisms occurs with acrylates, methacrylates, and styrene. Acrylamide can also be polymerized by a hydrogen transfer mechanism using a basic catalyst to form poly (B-alanine) or nylon-3, claimed to be a replacement for natural silk.

The reaction of acrylamide with formaldehyde to form N-methylolacrylamide is significant from a commercial standpoint:

$$CH_2O + CH_2{=}CHCONH_2 \xrightarrow[\text{aqueous}]{\text{pH 9.6}} CH_2{=}CHCONHCH_2OH$$

when it acidifies, N-methylolacrylamide will react with additional acrylamide to form N,N'-methylenebisacrylamide.

The reaction of sodium sulfite or bisulfite yields sodium-sulfopropionamide:

$$NaHSO_3 + CH_2{=}CHCONH_2 \longrightarrow NaO_3S\ CH_2CH_2CONH_2$$

This reaction is rapid when the sulfite ion is at room temperature, and because of this compound's low toxicity, the use of sodium sulfite as a scavenger for acrylamide monomer is recommended. The procedure consists of diluting the monomer with water at a 1.0 or greater dilution, adding a chelating agent, and then adding the sulfite.

4. ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

4.1. Summary and Conclusions

Acrylamide is a small organic molecule that is very soluble in water. It has an electrophilic vinyl group which may be attacked by nucleophiles. This characteristic is utilized to create various polymers and accounts for its widespread use. On the other hand, this same reactivity would permit acrylamide to react with biological macromolecules. Accordingly, studies of the absorption, distribution, metabolism, and elimination of acrylamide in organisms are important to elucidate its toxicity.

Available studies show that the pharmacokinetics and tissue distribution of acrylamide were not significantly affected by the dose administered or by the route administration. However, giving the chemical for 13 consecutive days slightly modified its distribution. The parent acrylamide was eliminated from tissues in a monophasic fashion, an effect attributed to metabolism, but the total radioactivity (labeled acrylamide and its metabolites) was cleared in a biphasic manner. The initial phase of the elimination curve was believed to result from the metabolism of acrylamide and its binding to macromolecules, while the terminal portion of this curve probably represented the clearance of metabolites from tissue depots and degradation of acrylamide-bound adducts.

After oral administration, acrylamide was rapidly and completely absorbed from the gastrointestinal tract. However, after dermal exposure to the intact animal, approximately 25% of

the applied dose penetrated the skin and was absorbed in 24 hours. After absorption, acrylamide quickly equilibrated throughout the body and did not concentrate in any tissue. The compound did, however, accumulate and persist in the red blood cells (RBC), presumably by reacting with sulfhydryl groups present in hemoglobin. After absorption, acrylamide was rapidly metabolized primarily by conjugation with glutathione, and over 60% of the applied dose was excreted in the urine within 24 hours. The major urinary metabolite of acrylamide, accounting for approximately 50% of the dose, was N-acetyl-S-(3-amino-3-oxypropyl)cysteine, a metabolic breakdown product of the acrylamide-glutathione adduct. Three unidentified non-sulfhydryl metabolites, comprising another 14%, were also found in the urine. In addition, small quantities of the chemical were eliminated in the urine as the parent compound (2%), via the lungs as CO_2 (4 to 6%) and as unidentified metabolites in the feces (6%). The remainder was bound to tissue components. Acrylamide has also been reported to bind to nucleic acids and proteins *in vivo* and DNA *in vitro*. This adduct formation may play a role in the toxicity of acrylamide.

4.2. Absorption

The uptake of acrylamide through the gastrointestinal tract of rats was rapid and complete based on the similar excretion profiles for 10 mg of acrylamide/kg whether the chemical was administered by intravenous (iv) injection or by gavage (Miller

et al., 1982). Absorption through rat skin, however, was less than complete. By comparing blood levels after iv or dermal administration of acrylamide, Ramsey et al. (1984) calculated that 25% of the applied doses (2 or 50 mg/kg) of acrylamide was absorbed through the skin. A recent study has confirmed and extended these results. Frantz et al. (1985) reported that 26% of a 0.5% aqueous solution of acrylamide was absorbed through the skin of rats in 24 hours and that, after washing of the skin, an additional 35% was still present in the skin. The data from in vitro experiments were similar (Frantz et al., 1985). Using excised skin preparations, it was shown that 67% (54% absorbed and 13% present in skin after washing) of applied acrylamide was either absorbed or available for absorption. Their preliminary data also suggested that residual acrylamide monomers present in polyacrylamide are absorbed to a greater extent than the acrylamide monomers in a 0.5% water solution.

4.3. Distribution and Pharmacokinetics

After iv or oral administration of varying doses of acrylamide (0.5 to 100 mg/kg) to rats, the ^{14}C-labeled chemical quickly distributed throughout the body (Hashimoto and Aldridge, 1970; Edwards, 1975; Miller et al., 1982; Ramsey et al., 1984). Approximately 12% of the label rapidly accumulated in RBC (Hashimoto and Aldridge, 1970; Miller et al., 1982) and high levels persisted for at least 10 days. This persistence has been postulated to result from the reaction of acrylamide with

sulfhydryl moieties present in hemoglobin (Hashimoto and Aldridge, 1970). Miller et al., (1982) reported that higher percentages of ^{14}C-label were found in muscle (48%), skin (15%), blood (12%) and liver (7%), while the neural tissues (brain, spinal cord, and sciatic nerve) contained less than 1% of the label. However, when the data were expressed as micromoles of acrylamide/gram of tissue, the concentrations of acrylamide in the tissues were similar. Accordingly, preferential bioconcentration of acrylamide and/or its metabolites in neural tissues did not occur and cannot account for its neurotoxic effects (Miller et al., 1982).

Acrylamide has also been reported to distribute readily in other species. For example, it was found in the blood, brain, heart, liver, kidney, and lungs of miniature swine and beagle dogs, with higher concentrations in the liver and kidney (Ikeda et al., 1985). Since these authors did not analyze skin, muscle, and other tissues, it is not possible to compare their results to those of Miller and coworkers (1982). In addition, autoradiographic studies have demonstrated similar distribution of acrylamide in male and pregnant female mice (Marlowe et al., 1986). It is worth noting that acrylamide crossed the placenta of rats, rabbits, dogs, pigs, and mice and was uniformly distributed in the fetuses (Ikeda et al., 1983, 1985; Marlowe et al., 1986).

The effects of multiple oral doses on tissue distribution have also been examined (Ramsey et al., 1984). When rats were

given acrylamide at 0.5 or 30 mg/kg for 13 consecutive days, the ratio of the label in the tissues at the two doses, except in the RBC, blood plasma, and testes, was proportional to the ratio of the doses administered. The RBC ratio for the two doses was 304, while the ratios in plasma and testes were 1,089 and 943, respectively (Table 4.1). These data have demonstrated that multiple doses of acrylamide do not greatly alter its distribution, except at these three sites.

A detailed study of the pharmacokinetics and distribution of acrylamide, both as the parent compound and as the total ^{14}C-label after an iv dose of 10 mg/kg, was conducted by Miller et al. (1982) in rats. The elimination of the parent acrylamide could be represented by a single compartment model. In the blood, the parent compound had a half-life of 1.7 hours and the clearance of the unmetabolized acrylamide from all other tissues, except the testes, was similar. The testes showed a delay in the time necessary to reach peak concentration; an event attributed to its fat content (Miller et al., 1982). After the peak was attained, the parent acrylamide was cleared in a manner similar to the other tissues (Miller et al., 1982; Fig. 4.1).

The distribution and elimination of the total ^{14}C-label, representing acrylamide and its metabolites, was slower than that of the parent acrylamide and was best represented by a biphasic curve (Fig. 4.2). In addition, four tissues (liver, kidney, fat and testes) demonstrated absorptive phases. Since no absorption phases were noted for the parent acrylamide in the liver and

Table 4.1. Concentration of ^{14}C activity in selected tissues (as ug equivalents ^{14}C-acrylamide per g) of rats after 13 daily oral doses of 0.05 mg or 30 mg 1,3-^{14}C-acrylamide per kg. Values are mean ± SD for 4 rats.

	ug Eg/g Tissue, 30 mg/kg	Mean ± SD 0.05 mg/kg	Ratio 30/0.05
Brain	53.52 ± 26.66	0.0834 ± 0.0065	641
Liver	87.74 ± 70.30	0.1438 ± 0.0017	610
Kidneys	70.43 ± 58.62	0.1291 ± 0.0160	546
Testes	67.14 ± 45.29	0.0719 ± 0.0070	948
Epididymes	70.60 ± 21.54	0.1343 ± 0.0176	526
Sciatic Nerve	54.00 ± 22.13	0.0856 ± 0.0079	631
Carcass	47.56 ± 15.71	0.0757 ± 0.0075	628
Skin	39.11 ± 14.31	0.0706 ± 0.0226	554
RBC	383.70 ± 111.65	1.2633 ± 0.0801	304
Plasma	16.45 ± 18.75	0.0151 ± 0.0039	1089
			647 ± 220

From: Ramsey et al., 1984 (used with permission).

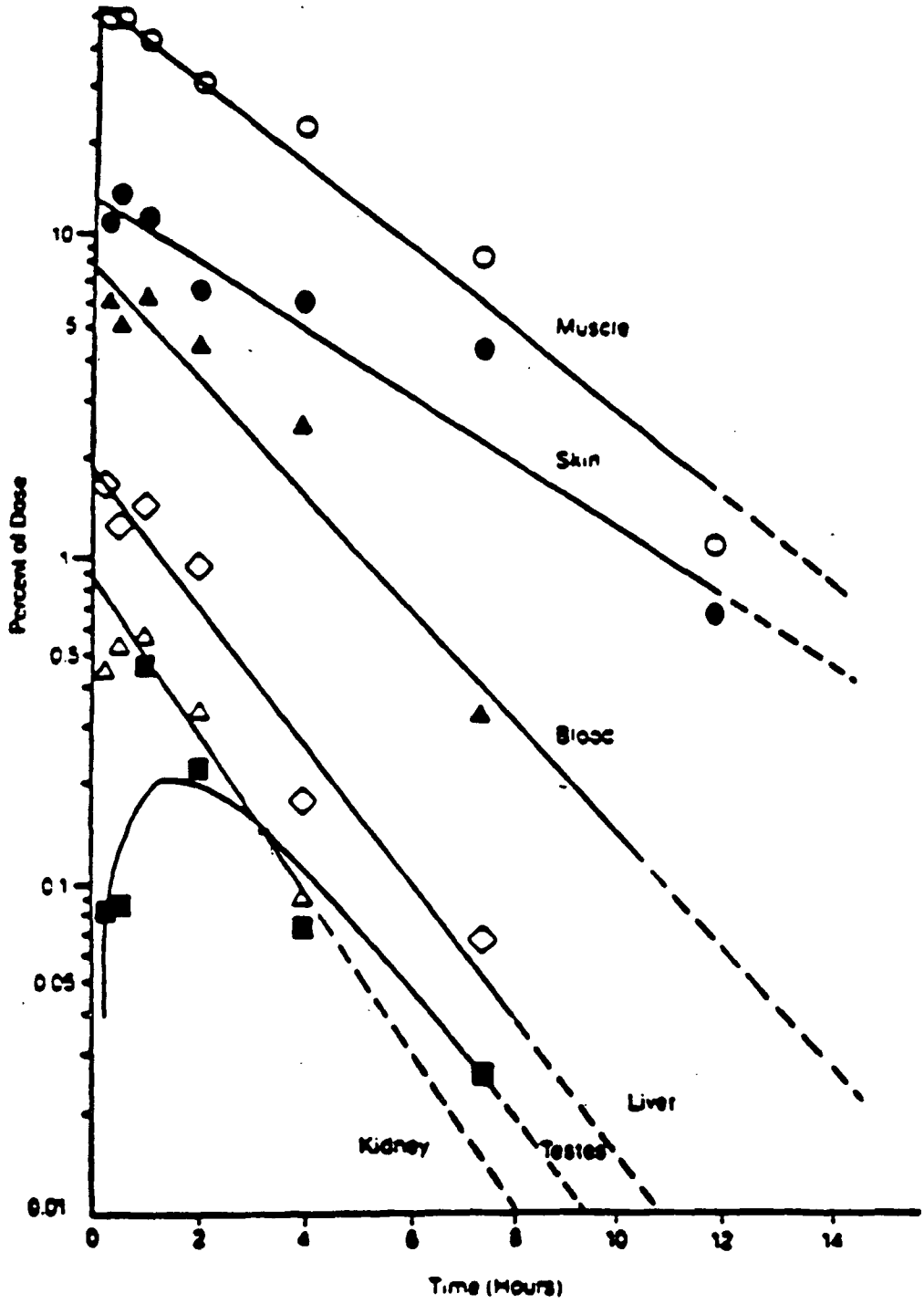


Figure 4.1. Elimination of Parent Acrylamide from Tissues After 10 mg/kg iv. Each symbol represents the mean percentage of total dose as parent obtained from three animals.

Adapted from Miller et al., 1982, with permission.

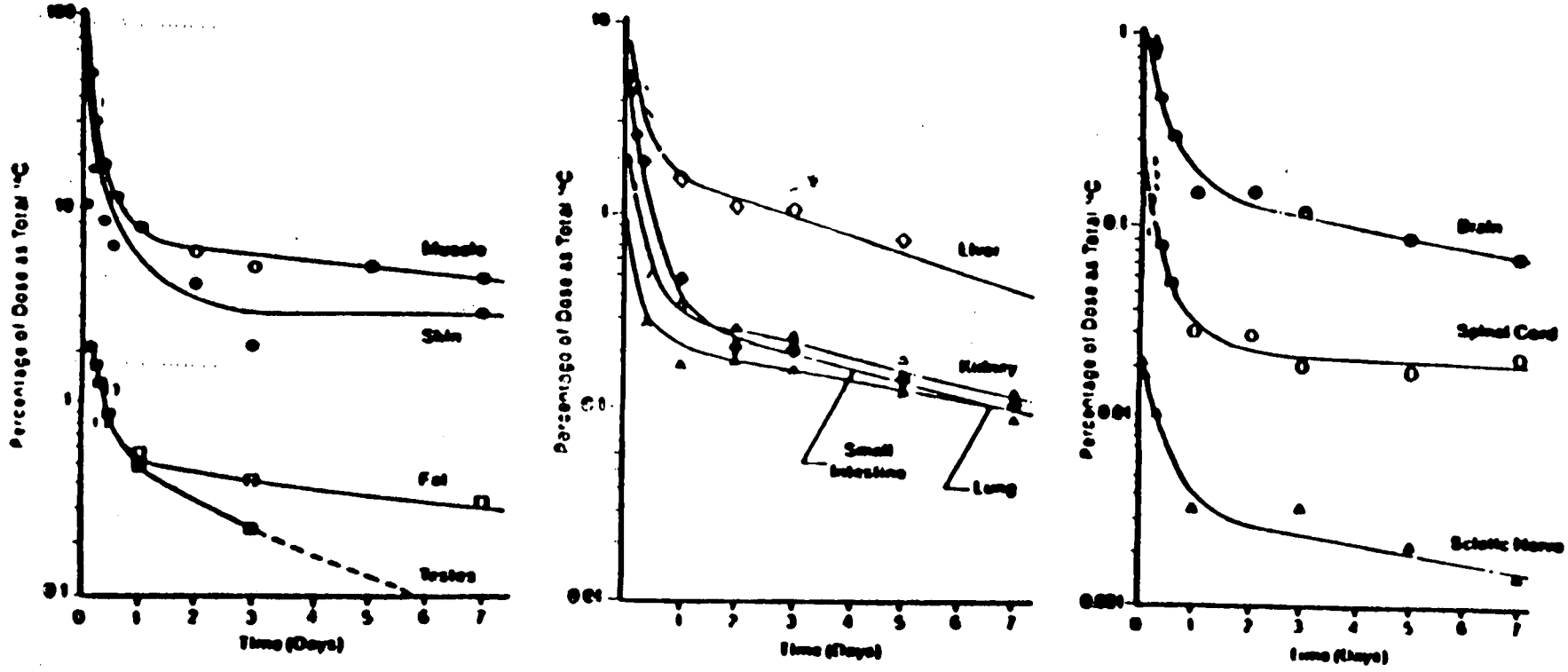


Figure 4.2. Tissue Distribution of Radiolabel Following 10 mg/kg Acrylamide Administered iv. Each symbol represents the mean percentage of total dose obtained from three animals.

Adapted from Miller et al., 1982, used with permission.

kidney, the increases in these two tissues were attributed to metabolite accumulation. The higher lipid content of the fat and testes and the polar nature of acrylamide were believed by Miller and coworkers (1982) to have delayed absorption in these two organs.

The initial portion (half-life of 5 hours) of the biphasic curve was attributed to the metabolism of acrylamide and the binding of its metabolites to biological macromolecules, since only 2% of the total dose of administered acrylamide was excreted as the parent compound. The terminal phase (half-life of 8 days) was thought to be the result of the release of acrylamide metabolites from tissue depots and the degradation of acrylamide-protein adducts (Miller et al., 1982). Support for these suppositions was provided by Ramsey et al. (1984). Using gas chromatographic/mass spectrometric analysis of plasma samples, their data indicated that the initial phase was due to the loss of the parent compound, while the latter phase resulted from the clearance of acrylamide metabolites.

Of particular interest is the very slow release of acrylamide and/or its metabolites from the testes (Fig. 4.2). Although the fat content of the testes may have decreased the uptake of the parent acrylamide (Fig. 4.1), it would not appear to account for its slow excretion. It is possible that the acrylamide is metabolized and binds to constituents of the testes. In this connection, the results of Marlowe et al. (1986) are relevant. They reported that radioactivity appeared in the

testes one hour after administration and by 9 hours had migrated to the seminiferous tubules and head of the epididymis. After 9 days, radioactivity remained only in the tail of the epididymis and in the epithelium of the penis. They correlated this movement with that of the spermatid. Further work is needed to confirm their suppositions.

4.4. Metabolism and Excretion

Conjugation with glutathione (GSH) appears to be a likely route for the biotransformation of acrylamide because several studies indicate that acrylamide depletes GSH (Hashimoto and Aldridge, 1970; Edwards, 1975; Srivastava et al., 1983) and that hepatic GSH-S-transferases catalyse the reaction of acrylamide and GSH (Dixit et al., 1981). Indeed, this has been shown to be the case. The major urinary metabolite of acrylamide is N-acetyl-S-(3-amino-3-oxypropyl)cysteine and accounts for approximately 50% of the applied dose (Miller et al., 1982; Ramsey et al., 1984). Three other unidentified, non-sulfhydryl urinary metabolites accounted for an additional 14% (Miller et al., 1982). When acrylamide was labeled at the carbonyl carbon, it was found that 4 to 6% of the label was eliminated as CO_2 (Hashimoto and Aldridge, 1970; Ramsey et al., 1984). Since 15% of the dose appeared in the bile within 6 hours, but only 6% was excreted in the feces, acrylamide (or its metabolites) underwent enterohepatic circulation (Miller et al., 1982).

Studies on the effects of altering microsomal mixed-function oxidase activity on the expression of acrylamide-induced neuropathy have given disparate results. For example, it has been reported that pretreatment of rats with phenobarbital (PB) or DDT (Kaplan et al., 1974) or mice with PB (Hashimoto et al., 1981) reduced or delayed the neurological dysfunction caused by acrylamide. On the other hand, it has also been reported that pretreatment of rats with PB or DDT decreased the time necessary for acrylamide-induced hindlimb paralysis (Srivastava et al., 1985). These differences do not appear to result from differences in experimental protocol. For example, Kaplan et al. (1973) using 40 and 60 mg/kg and Srivastava et al. (1983) using 50 mg/kg gave similar doses of acrylamide. In addition, both groups administered the drugs by ip injection and gave repeated daily doses of the inducer and acrylamide concurrently. However, there were differences in the animals. Kaplan et al. used male Holtzman rats (200 to 300 g), while Srivastava et al. used male Wistar rats (90 to 110 g). Thus, it is possible that strain and age differences played a role. Nevertheless, reconciliation of these reports awaits further experiments.

Similarly, the use of drug metabolism inhibitors has given results that are difficult to interpret. For example, SKF 525A has been reported to prevent acrylamide-induced enhancement of striatal dopamine receptor activity (Agarwal et al., 1981) and to increase the acute toxicity of acrylamide (Kaplan et al., 1973), while cobalt chloride has been shown to cause a significant delay

in the development of hindlimb paralysis (Srivastava et al., 1985). Presently, there is no correlation between the effects of modifying microsomal metabolism of acrylamide and the expression of its toxic effects. Accordingly, identification of those metabolic factors important in the toxic manifestations of acrylamide is not possible.

4.5. DNA Binding

In light of acrylamide's reported genotoxicity and carcinogenicity (see sections 7 and 8), information on the binding of this compound to DNA is of considerable interest. In this regard, it has been demonstrated that binding of acrylamide to DNA occurs in the lung, liver, testes, stomach, and skin of mice 6 hours after oral or dermal administration of acrylamide (Carlson and Weaver, 1985). Whether the parent acrylamide or a metabolite was responsible for the acrylamide-DNA adducts was not addressed. Recently, it has been shown that acrylamide can alkylate DNA in an *in vitro* system (Solomon et al., 1985). However, the yield of alkylated DNA, after 40 days of incubation, was low (approximately 10%) and the relevance of these *in vitro* binding studies to the *in vivo* DNA binding studies reported by Carlson and Weaver (1985) remains to be established. However, the fact that acrylamide has been shown to interact with genetic material may have implications for its genotoxic and carcinogenic effects.

5. NEUROTOXIC EFFECTS

Unlike the other health effects of acrylamide, the effects of acrylamide on the nervous system have been reviewed in several recent EPA reports and peer-reviewed journal papers including: the Office of Toxic Substances (USEPA, 1980); the Office of Drinking Water (USEPA, 1985), Tilson (1981), Howland (1985), Miller and Spencer (1985), and O'Donoghue (1985). Because of the availability of these thorough evaluations of acrylamide's neurotoxic effects, the present assessment focuses on a review of the results from a recent chronic drinking water study in rats, not previously reviewed (Johnson et al., 1984; 1985; Gorzinski et al., 1984) and an identification of the data to be used for risk assessment purposes.

5.1. Summary and Conclusions

Exposure to acrylamide by a variety of routes may produce serious neurological effects. Predominant among these effects in humans are sensory paresthesia of the hands and feet, muscle weakness, ataxia, decreased tendon reflexes, and other signs of peripheral nerve damage. Other effects, referable to the central nervous system, have also been reported in humans and include drowsiness, tremors, slurred speech, and hallucinations. The human reports of neurotoxicity also indicate that although most of the individuals recovered completely after the exposure was stopped, some severely affected persons did not appear to recover

completely following cessation of the exposure, indicating an irreversible effect.

Acrylamide destroys the most distal axons of both central and peripheral neurons and interferes with retrograde axonal transport. It also produces a number of biochemical effects that may or may not be relevant to its neurotoxicity. However, the precise mechanism of action by which acrylamide produces neurotoxicity is unknown.

The human data are quantitatively inadequate for risk assessment because few data on human exposure levels exist. Based on recent and older studies of prolonged exposure in rats, cats, and monkeys it appears that prolonged exposure to 1 mg/kg/day or more causes neurotoxic effects.

5.2. Human Case Studies

The effects of acrylamide in humans were reviewed by EPA in 1980 (USEPA, 1980). There are 53 reported cases of acrylamide toxicity, all but 5 relate to occupational exposure. In these occupational cases, dermal exposure predominates, with some inhalation exposure and potential oral exposure secondary to exposure by the dermal route. As is typical in such cases, the exposure levels are only qualitatively known. However, the severity of effects in these reports does seem to be a function of exposure duration.

Table 5.1. Signs and Symptoms from Human Case Reports.

The signs and symptoms of acrylamide exposure from these case reports are summarized in Table 5.1. The early signs and symptoms of exposure include: skin peeling and other skin changes, numbness/tingling of the extremities, coldness of the skin, excessive sweating, bluish-red skin color, and muscle weakness. These are generally followed by fatigue, confusion and other psychological effects, gastrointestinal problems, and weight loss. These signs typically precede the dramatic symptoms of overt peripheral nerve dysfunction (i.e., ataxia and weak or absent tendon reflexes). These may be followed by an inability to stand, body tremors, slurred speech, difficulty in swallowing and other signs. In general, victims showed complete recovery, although not always. For example, Garland and Patterson (1967) described poisoning in six men who had worked for periods varying from 4 to 60 weeks in factories manufacturing acrylamide-based flocculants. Individuals with relatively mild symptoms recovered completely within 2 to 12 months after exposure to acrylamide ceased. Severely affected persons did not appear to recover completely following cessation of exposure. The clinical picture in these individuals suggested peripheral neuropathy and mid-brain disturbance. In summary, the human case reports present evidence of both central and peripheral nervous system effects following short and long-term exposure to acrylamide. In principle, central effects are much more likely to be irreversible if neural damage takes place and are, therefore, of greater concern.

In only one human case study can exposure be estimated. Igisu et al. (1975) described the health effects of acrylamide in a Japanese family of five individuals that was exposed through ingestion and external use of well water contaminated with acrylamide seeping from a grouting operation. The well water was analyzed by gas chromatography and found to contain 400 mg/l of acrylamide. Assuming a daily water consumption of 2 liters and a body weight of 70 kg for the adults, the exposure corresponds to a daily dose of about 11.4 mg/kg. Symptoms of toxicity developed about one month after the grouting operation and the mother, father, grandmother were hospitalized. Each exhibited similar symptoms consisting of marked rhinorrhea, coughing, dizziness, irrational behavior, poor orientation and memory, as well as visual, auditory, and tactile hallucinations followed by slurred speech and unsteadiness in walking. The two children, who were at school much of the time and apparently did not consume as much well water as the adults, had only mild symptoms. Both children recovered within two weeks, and the adults recovered within two to four months. These effects and doses correspond fairly well to data from prolonged exposure in cats and monkeys. Despite the data from this one case, the human data are inadequate for quantitative risk assessment purposes.

5.3. Animal Studies

5.3.1. Acute Exposure Studies

Agrawal et al. (1981) studied the effect of a single oral dose of acrylamide on neurotransmitter levels in the brain. Six-week old male Fischer 344 (F344) rats were administered a single dose of 0, 25, 50, or 100 mg/kg by gavage (six to eight animals per dose). The rats were killed 24 hours later and specific brain regions were removed for analysis. Significantly increased binding of ^{3}H-spiroperidol was observed in striatal tissue at all doses of acrylamide ($P<0.05$). In animals that received 100 mg/kg, there was significantly increased binding of ^{3}H-strychnine in the medulla and of serotonin in the frontal cortex ($P<0.05$). No changes were observed in muscarinic receptors in the striatum, benzodiazepine receptors in the frontal cortex or GABA receptors in the cerebellum.

Miller et al. (1983) studied the effect of single doses of acrylamide on retrograde axonal transport in rats. Animals were dosed with acrylamide by ip injections (five animals per dose). Retrograde transport was assessed by movement of ^{125}I-labeled nerve growth factor (injected into front foot pads) toward spinal ganglia. Acute acrylamide doses of 25 mg/kg or higher resulted in significant inhibition of retrograde transport, but doses of 15 mg/kg or less did not. Multiple injections of 15 mg/kg/day caused inhibition of transport after five doses (cumulative dose of 75 mg/kg). Hindlimb foot-splay was not affected until ten

doses (cumulative dose of 150 mg/kg) had been administered, indicating that retrograde transport is a more sensitive indicator of acrylamide-induced neurotoxicity than the foot-splay test. The results of this study suggest that a single dose of 15 mg/kg is a NOEL and a single dose of 25 mg/kg is a LOEL for acute neurotoxicity. Although this study was conducted using an ip injection, there are data to suggest that these results can be used to assess the risk from other human exposure pathways. The results presented in the metabolism section indicate that acrylamide is rapidly and completely absorbed from the gastrointestinal tract and that metabolism, distribution, and clearance of acrylamide are similar following either oral or parenteral administration. For this reason it is considered acceptable to employ the NOEL established by Miller et al. (1983) for risk assessment of acute exposures.

5.3.2. Recent Chronic Exposure Studies

A chronic acrylamide exposure study with rats (F344) has recently been completed. This study included two distinct parts: the main part was a 2-year bioassay with traditional gross and histopathological observations of the nervous system (among others), and the secondary part was an electron microscopy (EM) portion designed to provide a more powerful evaluation of histological changes to the nervous system.

In the main part of the bioassay (Johnson et al. 1984; Gorzinski et al. 1984), 90 rats/sex/dose were exposed to either

0, 0.01, 0.1, 0.5, or 2.0 mg/kg/day of acrylamide via the drinking water for up to 2 years. Ten rats/sex/dose were sacrificed after 6, 12, or 18 months of exposure. In addition to the traditional toxicological measures taken (weight, food intake, gross observations, etc.), those measures most relevant to neurotoxicity were the clinical observations and the histopathology of the nervous system. Approximately monthly, selected subjects were examined outside the cage for neuromuscular coordination. At sacrifice, rats were anesthetized and decapitated. The brain was preserved in neutral, phosphate-buffered 10% formalin. Three pairs of peripheral nerves: sciatic, femoral, and brachial plexus were dissected from 10 rats/sex/dose. Three sections of the spinal cord, cervical, thoracic, and lumbosacral were prepared, and 5 coronal sections of the brain were routinely processed. Processing was standard, and hematoxylin and eosin stained sections were prepared. Nerves were graded on a 4-point qualitative scale, from very slight to severe damage. For histopathologic observations, statistical comparisons were made with Fisher's Exact Test between groups and their cumulative incidence.

High dose (2.0 mg/kg) males showed a reduced body weight beginning at roughly 3 months of exposure and continuing throughout the study. Male rats given 0.5 or 2.0 mg/kg that were sacrificed at 18 months had significantly decreased body weights. Between 21 and 24 months, there was increased mortality for both sexes at 2.0 mg/kg. No changes were reported at any time in

gross behavior or motor function that might be related to peripheral nerve or spinal cord lesions. The data from the interim sacrifices were described by Gorzinski et al. (1984).

At the 6-month sacrifice, no changes were seen in histopathological measure in peripheral nerves, spinal cord, or brain sections.

At 12 months, both males and females exposed to 2.0 mg/kg showed increases in degeneration of the tibial nerve. These changes consisted of focal swelling of fibers with fragmentation of myelin and the axon and the formation of digestion chambers. No changes in spinal cord or brain were seen.

At 18 months, changes in tibial nerve were again seen in males and females receiving 2.0 mg/kg as well as similar effects in the brachial nerve. In addition, there was some increase in very slight changes in the cervical cord of males given and in the thoracic cord of females given 2.0 mg/kg.

Data from the terminal sacrifice and cumulative results are described by Johnson et al. (1985). In these data there appears to be a trend in the incidence of tibial nerve degeneration data in males as indicated by higher degeneration ratings, but it is not a large effect and apparently, was not statistically significant. There also appears to be an increase in slight degeneration of tibial nerve of females particularly at terminal sacrifice. It does not, however, appear to be dose-dependent (i.e., occurs in the same frequency in all dose groups), and apparently lacks statistical significance. There is a

significant linear trend in the brachial nerve data for males; an increase in rating of very slight damage in the 0.1 and 0.5 mg/kg/groups (dose-ratings were: 0-3; 0.01-2; 0.1-6; 0.5-8; 2.0-5). Females showed some effect, contrary to the author's assertions, in the thoracic spinal cord, namely, slight degeneration of the white matter at the 2.0 mg/kg dose. These were statistically different from controls.

In summary, histopathological changes were seen in the tibial nerves of rats exposed to 2.0 mg/kg after 12 and 18 months. At 18 months, changes in brachial nerve were seen as well as slight changes in the cervical cord of males given 2.0 mg/kg and the thoracic cord of females given 2.0 mg/kg. Thoracic cord changes in 2.0 mg/kg females were again seen at the terminal sacrifice. For this portion of the study, then, with regard to neurotoxicity, 0.5 mg/kg may be viewed as a NOEL for both central and peripheral nerve damage.

In the EM portion of the chronic rat (F344) bioassay with acrylamide, Johnson et al. (1985) examined the tibial nerves of 3 rats/sex/dose after 3, 6, 12, 18, and 24 months of exposure. The dose levels in the drinking water, 0, 0.01, 0.1, 0.5, and 2.0 mg/kg/day, were the same as in the main part of the bioassay. Since females showed little effect in comparison to males by 12 months, only male rats were examined after 18 and 24 months of exposure.

Subjects were anesthetized and perfused with saline and glutaraldehyde-formaldehyde fixative. The peripheral nerve was

then removed, washed in phosphate buffer, fixed with osmium tetroxide (1%), dehydrated, and embedded in plastic. Thin sections (600-700 angstroms) were made from cross-sectional and longitudinal blocks and stained with lead citrate and uranyl acetate. Sections were examined and evaluated with two methods (one qualitative and one quantitative) by technicians blind to the nature of treatment of any given section. The qualitative evaluation method involved a five point rating scale, from essentially none to severe for rating "overall degree of involvement" in a section. The quantitative method consisted of counting myelinated fibers and characterizing changes in them in terms of: axolemmal invaginations with or without inclusions (2 categories), axonal degenerative changes, Myelin/schwann cell degenerative changes, and regenerative changes.

No pronounced effects on body weight, food and water intake, or gross pathology were noted for these rats, although this may have been due to the small number of subjects since changes in these parameters were seen in the main part of this bioassay, as discussed previously. Light microscopic histopathology on these rats revealed changes similar to those in the main part of the bioassay, namely increases in degenerative changes of peripheral nerve after 12 months or more at the highest dose of 2.0 mg/kg, as well as increases in neuropathy of control and treated animals after 12 or more months.

The major finding from the EM examinations was an increase in axolemmal invaginations into the axoplasm of nerves (infolding

of the axon membrane into the axon plasma) of male rats given 2.0 mg/kg/day, a possible early sign of nerve damage. After 3 months of exposure, these changes were seen on cross sections only, but after 6 months and 12 months both longitudinal and cross sections showed overtly increased percentages of affected fibers. Some signs of increased incidence of axonal and myelin degeneration were seen after 12 months in males as well. After 18 and 24 months, there were more fibers qualitatively judged to be more seriously affected, but they were not clearly treatment related. The quantitative measures showed no consistent effect of treatment. There were no changes seen in male animals at lower doses or in females that could be attributed to treatment. This may have been due to the small number of subjects in the study, which made analysis of the results difficult.

Despite these limitations, the data and the study are thorough and sound and may be used to conclude that 2.0 mg/kg/day for as little as 3 months produces neuropathological changes in peripheral nerves. Thus, for this part of the study, 0.5 mg/kg/day is a subchronic (3-12 month) NOEL. The quantitative measures showed no effect after 18 and 24 months of exposure, but, as the authors note, this may have been due to the small size of nerve area and number of animals examined, and is inconsistent with light microscopic changes seen in other rats in the main part of the bioassay. Thus, 2.0 mg/kg/day should prudently not be regarded as a chronic no effect level for

acrylamide based on the quantitative measure. The 18 and 24 month data are indeterminate.

5.3.3. Previous Chronic Exposure Studies

The key long-term animal studies are summarized in Table 5.2. These were drawn from the larger literature summarized in Table 5.3 from Conway et al. (1979) and reviewed in USEPA (1980) and from the more recent studies. Studies were chosen because they showed effects (LOEL) or no effects (NOEL) at the lowest exposure levels. To the extent verifiable, all of these studies appear to be valid (i.e., well-conducted and scientifically sound).

Burek et al. (1980) reported that pathological changes were seen under electron microscopy in rats given 1 mg/kg for 90 days. These changes consisted of axolemmal invaginations and organelles or dense bodies in sections of the sciatic nerve.

Table 5.2. Key Animal Studies of Subchronic and Chronic Exposure to Acrylamide (All studies here except the 2 noted used the oral route of administration).

Reference	Species (Route)	Exposure Duration	mg/kg NOEL	mg/kg LOEL
Burek et al. 1980	Rats	90 days	0.2	1.0
Johnson et al. 1985	Rats	1 year	0.5	2.0
Johnson et al. 1984	Rats	2 year	0.5	2.0
Hamblin 1956	Cats (i.v.)	180 days		1.0
Kuperman 1958	Cats (i.p.)	125 days		1.0
McCollister et al. 1964	Cats	1 year	0.3	1.0
McCollister et al. 1964	Monkeys	1 year	1.0	3.0 (10.0)
Spencer 1979	Monkeys	1 year	2.0	3.0
Schaumberg et al. 1982	Monkeys	1.5 years		1.0

Table 5.3. Acrylamide Doses Producing Early Signs of Peripheral Neuropathy in Various Mammals.

Organism	Route	Dose, schedule	Days to Initial effect (No. of doses)	Total administered dose (mg/kg)	Reference
Rats (adult)		100 mg/kg, 2 doses/wk[c]	21(6)[a]	600	Fullerton and Barnes, 1966
	Oral	100 mg/kg, 1 dose/wk	42(6)	600	
		100 mg/kg, 1 dose/2 wk	210(15)	1500	
	Ip	75 mg/kg, 1 dose/day	4.6[b]	345	Kaplan and Murphy, 1972
	Ip	50 mg/kg, 3 doses/wk	18(7-8)	350-400	Suzuki and Pfaff 1973
	Ip	50 mg/kg, 1 dose/day	6.4[b]	320	Kaplan and Murphy, 1972
	Oral	40 mg/kg/day[c]	14	560	McCollister et al. 1964
	Ip	40 mg/kg, 1 dose/day	6.7[b]	268	Kaplan et al., 1973
	Oral	30 mg/kg/day[c]	21	630	McCollister et al. 1964
	Ip	30 mg/kg, 1 dose/day	10.7[b]	321	Kaplan et al., 1973
	Oral	25 mg/kg, 5 doses/wk	28(20)	500	Fullerton and Barnes, 1964
	Ip	25 mg/kg, 1 dose/day	16.8[b]	420	Kaplan and Murphy 1972
	Oral	9 mg/kg/day[c]	56[d]	504	McCollister et al. 1964
Cats	Ip	50 mg/kg, 1 dose/day	2(2)	100	Kuperman 1958
	Oral	20 mg/kg, 1 dose/day	14-21	280-420	Loewing and Ribelin, 1969

Source: Conway et al. (1979)

[a]Signs of intoxication probably appeared earlier than noted.

[b]Signs of intoxication based on electrorod measurements.

[c]Acrylamide mixed with food. Dose estimated by McCollister and coworkers, 1964.

[d]Effect noted in only 1/20 exposed animals.

Table 5.3. Acrylamide Doses Producing Early Signs of Peripheral Neuropathy in Various Mammals (continued).

Organism	Route	Dose, schedule	Days to Initial effect (No. of doses).	Total administered dose (mg/kg)	Reference
(cont.)	Ip	20 mg/kg, 1 dose/day	5	100	Schaumberg et al. 1974
	Ip	10 mg/kg, 1 dose/day	13-16	130-160	Schaumberg et al. 1974
	Sc	10 mg/kg, 1 dose/day	17-22	170-220	_____ ,1969
	Oral in chow	3 mg/kg, 5 doses/wk	68	144	McCollister, et al., 1964
	Oral in water	3 mg/kg, 1 dose/day	70,163	210,409	Schaumburg, et al. 1974
	Ip	1 mg/kg, 5-6 doses/wk	125	100	Kuperman 1958
	Iv	1 mg/kg, 5 doses/wk	180	130	Hamblin 1956
Dogs	Oral	15 mg/kg, 1 dose/day	21[a]	315	Thomern et al. 1974
	Oral	10 mg/kg, 1 dose/day	28-35[a]	280-350	Hamblin 1956
	Oral	5 mg/kg, 1 dose/day	21[a]	105	Thomern et al. 1974
Primates	Oral in fruit	20 mg/kg, 1 dose/day	16	320	Hopkins, 1970
	Oral in fruit	25 mg/kg, 1 dose/day	42	630	Hopkins, 1970
	Oral in fruit	10 mg/kg, 1 dose/day	42-97	420-970	Hopkins, 1970
	Oral in water	10 mg/kg, 49 doses/ 69 days	48	340	McCollister, et al., 1964

Source: Conway et al. (1979)
[a]Signs of intoxication probably appeared earlier than noted.
[b]Signs of intoxication based on electrorod measurements.
[c]Acrylamide mixed with food. Dose estimated by McCollister and coworkers, 1964.
[d]Effect noted in only 1/20 exposed animals.

These changes were not apparent in rats that were allowed to recover for one month prior to sacrifice. No effects were seen in rats given 0.2 mg/kg.

Three studies in cats show effects after exposure to 1 mg/kg. Hamblin (1956) and Kuperman (1958) found neurological (motor) signs in cats given iv or ip injections of 1 mg/kg for 125-180 days. McCollister et al. (1964) gave cats doses of 0.1, 0.3, 1.0, 3.0, or 10.0 mg/kg in the food, 5 days/weeks, for a year. Cats given 10 mg/kg first showed signs after 26 days, those given 3 mg/kg showed weakness in hind limbs after 68 days, and one of the 2 cats given 1.0 mg/kg showed hind limb weakness after 240 days. No histopathology changes were seen at any dose. Lower dose animals died of spontaneous disease, so there is no NOEL for this study other than the one cat that showed no effects after a year at 1.0 mg/kg.

McCollister et al. (1964) gave acrylamide orally to one female monkey/dose at between 0.03 and 10 mg/kg for one year. Monkeys given 1 mg/kg or less showed no effects. The monkey given 3 mg/kg showed decreased pupillary and patellar reflexes, and the monkey given 10 mg/kg showed hindlimb weakness after 29 doses. Therefore, this study provides a LOEL of 3 mg/kg and a NOEL of 1 mg/kg.

Spencer (1979) found minor histopathological changes in the spinal cord of a Rhesus monkey given 3 mg/kg of acrylamide orally for 49 weeks. Monkeys given 1 or 2 mg/kg for one year and one monkey given 0.5 mg/kg for about 1.5 years showed no effects.

Also, Schaumberg et al. (1982) reported that monkeys given 1 mg/kg of acrylamide for 18 months showed changes in the somatosensory evoked response.

Finally, the work of Merigan and his colleagues on the effects of acrylamide on the visual system should be noted. They found (Merigan et al., 1982, 1985; Eskin et al., 1985) that macaque monkeys given 10 mg/kg orally for 6-10 weeks showed temporary decrements in evoked potentials, and flicker fusion thresholds, while acuity measures showed only partial recovery up to 90 days after exposure. Pathological changes were seen in the distal optic tract (axonal swelling) and the lateral geniculate. These results, the authors suggest, show the early occurrence of potentially irreversible changes in the nervous system. While the dose levels are relatively high at 10 mg/kg and did indeed produce weight loss and ataxia, they make clear that the central effects of acrylamide also include potentially irreversible visual changes.

5.4. Data for Risk Assessment

While there are a variety of effects on the nervous system from chronic acrylamide exposures, those that are most serious, widely studied, and appear most prominent are the effects on motor systems in peripheral nerves and the spinal cord. Because the human data are primarily limited to case reports of unquantified exposure levels, a quantitative risk assessment for neurotoxicity must rely on the best available animal data. Also,

the available data are inadequate for estimating population sensitivity or relative species sensitivity to extrapolate from the animal data to human exposure situations. In general, the available studies reveal no peculiarities in the sensitivity of exposed groups, although most studies are limited by design and group size to elucidate such issues. Most of the species tested for these effects showed a sensitivity in the same general exposure range, which provides some confidence in making interspecies extrapolations.

The best animal studies for evaluating chronic acrylamide exposures are summarized in Table 5.2. The lowest LOEL reported for three species is 1 mg/kg/day. For cats and rats, effects at this level were seen after only 3 to 4 months of exposure. Thus, 90 days or more of exposure to 1 mg/kg/day is associated with neuropathological effects. This is generally consistent with the one quantified human exposure report (Igisu et al., 1975), where dramatic effects were observed at about 10 mg/kg/day after less than 1 month of exposure.

The no effect levels (NOELs) range from 2.0 to 0.2 mg/kg/day from the best available data. For each species tested, however, the NOELs are slightly different. For rats, 0.2 mg/kg/day for 3 months is the best NOEL (from electron microscopy study). Using light microscopy, however, a 2-year NOEL of 0.5 mg/kg/day was observed. Although a 2-year study, it was not judged the best because the NOEL was found with light microscopy, a fairly insensitive measure of structural integrity. For cats the NOEL

is 0.3 mg/kg/day for 1 year and for monkeys the NOEL is less than 1.0 mg/kg/day for 1.5 years.

5.5. Mechanisms of Neurotoxic Action

The current status of our knowledge concerning the biochemical mechanism of acrylamide's neurotoxicity has recently been reviewed by Miller and Spencer (1985), O'Donoghue (1985), and others.

Two potential mechanisms for acrylamide's peripheral neurotoxicity are under investigation. One involves the binding of acrylamide to neurofilaments and the other describes the inhibition of neural enzymes needed for energy production. Recent work lends support to each hypothesized mechanism. In a study by Sickles (1987), acrylamide demonstrated significant reductions in NADH-tetrazolium reductase (TR) activity in ligated axons, Purkinje neurons, and dorsal root ganglion neurons. In contrast, no significant reductions in activity were noted in non-neural tissue enzymes. Methylene-bis-acrylamide which is non-neurotoxic had no effect on enzyme activity levels except in Purkinje neurons. Sickles (1987) concluded that the data support, in general, the hypothesis that specific inhibition of neural NADH-TR activity by acrylamide is the primary site of action in producing neuropathy. On the other hand, binding to cytoskeletal elements of the nervous system has been reported by Lapadula et al. (1986). A series of *in vivo* and *in vitro* experiments were done using mice and brain microtubule and spinal

cord cytoskeletal preparations from rats. Binding to cytoskeletal elements occurred in the in vitro studies. In the in vivo studies, binding to cytoskeletal proteins was similar to that in the in vitro studies and occurred in all neural tissues. According to the authors, these data demonstrate that acrylamide may produce neuropathy by binding directly to cytoskeletal elements.

Acrylamide has been shown to interfere with retrograde axonal transport. How these biochemical effects singly or in combination relate to axonopathy needs further investigation before the mechanism of acrylamide's neurotoxicity can be elucidated.

6. DEVELOPMENTAL AND REPRODUCTIVE EFFECTS

6.1. Summary and Conclusions

Acrylamide produces both fetal and postnatal developmental effects in mouse and rat offspring following administration to pregnant dams. It produces neurotoxic effects (tibial and optic nerve degeneration) in the neonates at levels that are not toxic to the dam. In addition, acrylamide changes intestinal enzyme levels in the fetus at dose levels where no maternal toxicity is apparent, but the toxicological significance of these changes is not clear. There is concern for the conceptus following maternal exposure during gestation since these data show that acrylamide can have a direct effect on the conceptus. A thorough teratology study would provide needed data on the structural teratogenic effects of acrylamide on the conceptus. Due to the nature of acrylamide toxicity, a state-of-the-art behavioral teratology study is also needed to provide information on effects in the neonate.

Acrylamide also has an adverse effect on reproduction. A dominant lethal effect and decreased copulatory performance have been observed in rats. In one assay, rat testosterone levels were depressed, but the functional significance of this finding is uncertain. In the mouse, decreased fertility was observed following exposure of male mice to acrylamide and an increased resorption rate resulted from the exposure of male or female mice. Degeneration of testicular epithelial tissue and a dominant lethal effect have also been observed in other mouse

studies. The available data indicate that acrylamide can act directly on the reproductive system, rather than indirectly through stress or other systemic effects.

Developmental toxicity data indicate a lowest observed effect level (LOEL) of 20 mg/kg/day. Since this was the lowest dose level tested in the study, a no observed effect level (NOEL) was not established (Table 6-1). The LOEL and NOEL for reproductive effects (Table 6-2), are 8.8 and 4.2 mg/kg/day, respectively.

6.2. Developmental and Female Reproductive Effects

Several investigators have described the effects of acrylamide on the developing conceptus. Pregnant rats (Porton strain) were given acrylamide either as powder in the diet or by iv injection (Edwards 1976). In Group I, 8 rats were given 200 ppm acrylamide in the diet (about 20 mg/kg/day) from the day of mating until parturition. In Group II, 6 rats were given 400 ppm (about 40 mg/kg/day) acrylamide in the diet from the day of mating until gestation day 20, and then were subjected to Caesarean section. In Group III, 4 rats were given a single iv dose of acrylamide (100 mg/kg) on day 9 of gestation. The only difference between the control and Group II was a slight decrease

Table 6-1

Summary of Acrylamide

Developmental and Female Reproductive Toxicity

STUDY TYPE (Ref)	Species, Route dose levels	Maternal LOEL	Maternal NOEL	Developmental LOEL	Developmental NOEL	Observed Effects Maternal	Observed Effects Developmental
Developmental Toxicity (Edwards, 1976)	Porton Rat Oral-diet 0 ppm 200 ppm or 20 mg/kg/d gd 0-22 400 ppm or 40 mg/kg/d gd 0-20 100 mg/kg (i.v.) gd 9	200 ppm	----	----	400 ppm	Ataxia; Abnormal gait	None. It was shown, however, that acrylamide readily crosses the placenta.
Developmental Toxicity (1-Gen. Repro.) (American Cyanamid, 1980)	Sprague-Dawley Rat Oral-diet 0, 25, 50 ppm or 0, 2.5, 5.0 mg/kg/d 3 weeks prior to mating gd 1-19	50 ppm	25 ppm	dose unspecified	dose unspecified	Decreased body weight gain; slight alopecia Nerve fiber degeneration in sciatic and optic nerves	Wallerian degeneration of tibial nerve, unilateral optic nerve degeneration (dose level unspecified)
Reproduction and Fertility (Zenick et al., 1986)	Long Evans Rat Oral-drinking water 0, 25, 50, 100 ppm	50 ppm	25 ppm	50 ppm	25 ppm	decreased body weight (50 ppm); decreased fluid intake (50 ppm); increased hindlimb splaying (100 ppm);	decreased birth weight Decreased body weight gain thru day 42

Table 6-1 (continued)

Summary of Acrylamide

Developmental and Female Reproductive Toxicity

STUDY TYPE (Ref)	Species, Route dose levels	Maternal LOEL	Maternal NOEL	Developmental LOEL	Developmental NOEL	Observed Effects Maternal	Observed Effects Developmental
Developmental/ Neonatal (Walden et al., 1981)	Fischer 344 Rat Oral-gavage 20 mg/kg/d, gd 7-16	----	----	2.0 mg/kg	----	None reported	Changes in various intestinal enzyme levels measured in the neonate (see text).
Two-Generation Reproduction (Nalco Chemical Co. 1987)	Fischer 344 Rat Oral-drinking water 0, 0.5, 2.0, 5.0 mg/kg/d for 10 weeks throughout gestation and lactation, 11 weeks for second generation.	2.0 mg/kg	0.5 mg/kg	5.0 mg/kg	2.0 m/kg	Increased peripheral neuropathy Decreased body weight Decreased body weight gain Decreased No. of litters (fecundity index) Increased pre-implantation loss (5.0 mg/kg/d)	Increased resorptions per litter Decreased litter size

Table 6-1 (continued)

Summary of Acrylamide

Developmental and Female Reproductive Toxicity

STUDY TYPE (Ref)	Species, Route dose levels	Maternal LOEL	Maternal NOEL	Developmental LOEL	Developmental NOEL	Observed Effects Maternal	Observed Effects Developmental
Reproductive Toxicity Toxicity Assay (Sakamoto & Hashimoto, 1986)	ddY Mouse Oral-drinking water 0-5mM, 4-6 weeks.	1.2mM or 0.447 mg/kg/d	0.9 mM or 0.335 mg/kd/d	----	1.2mM	Increased No. resorptions per dam	None reported

Summary of Acrylamide

Male Reproductive Toxicity

Table 6-2

STUDY TYPE (Ref)	Species, Route and Dose levels	LOEL	NOEL	Observed Effects
Dominant Lethal and (2-Gen. Repro.) (Nalco Chemical Co., 1987)	Fisher 344 Rat Oral-drinking water 0, 0.5, 2.0, 5.0 mg/kg/day for 10 weeks, 11 weeks for second generation	2.0 mg/kg/d	0.5 mg/kg/d	Increased peripheral neuropathy Decreased body weight Decreased body weight gain Decreased No. of litters (fecundity index) Increased pre-implantation loss (5.0 mg/kg/d)
Testicular Effects (Hashimoto et al., 1981)	ddY Mouse Oral-gavage 0, 35.5 mg/kg 2/wk for 8-10 wk. Av. daily dose = 10.1 mg/kg	35.5 mg/kg	---	Degeneration of testicular epithelia Weakness and ataxia in hind limbs
Dominant Lethal Smith et al., (1986)	Long Evans Rat Oral-drinking water 0, 15, 30, 60 ppm or 0, 1.5, 2.8, 5.8 mg/kg/day for 80 days	2.8 mg/kg/d	1.5 mg/kg/d	Increased pre-implantation loss (high dose only) Increased post-implementation loss

Summary of Acrylamide

Male Reproductive Toxicity

Table 6-2 (continued)

STUDY TYPE (Ref)	Species, Route and Dose levels	LOEL	NOEL	Observed Effects
Reproductive Toxicity Assay (Sakamoto & Hashimoto, 1986)	ddY Mouse Oral-drinking water 0.5mM, 4-6 wks 4.2, 8.8, 13.1, 17.4 mg/kg/day for ___ ___ 18.7 mg/kg/day for ___ ___	8.8 mg/kd/d	4.2 mg/kd/d	Decreased fertility rate Decreased No. fetuses/dam Increased No. resorptions/dam
Reproduction and Fertility (Zenick et al., 1986)	Long Evans Rat Oral-drinking water male: 0, 50, 100, 200 ppm or 0, 4.2, 7.9, 11.6 mg/kg/day for 10 weeks	100 ppm	50 ppm	Decreased copulatory performance increased hindlimb splaying
Dominant Lethal (Sublet et al., 1986)	Long Evans Rat Oral-drinking water 0, 5, 15, 30, 45, 60 mg/kg/d for 5 days	30 mg/kg/d	----	Increased pre-implantation loss; Increased post-implantation loss; Effects seen primarily in weeks 1-3 post mating.

Summary of Acrylamide

Male Reproductive Toxicity

Table 6-2 (continued)

STUDY TYPE (Ref)	Species, Route and Dose levels	LOEL	NOEL	Observed Effects
Testosterone Assay (Ali et al., 1983)	Fischer 344 Rat Intraperitoneal 0, 10, 20 mg/kg/ day for 20 days	20 mg/kg	10 mg/kg	Dose-dependent decrease of testosterone and prolactin
Dominant Lethal Shelby et al., 1986)	C3H X101 hybrid mouse i.p. 125 mg/kg or 5 X 50 mg/kg/day	50 mg/kg/d	----	Increased post-implantation loss
Spermhead Abnormality (EPA, unpublished data)	B6C3F1 mouse 25, 50, 100 mg/ kg/day X 5 days	100 mg/kg/d	----	Decreased testes: body wt. ratio (100 mg/kg/d) Increase incidence of spermhead abnormalities.

in fetal weight of the latter which might be attributed to the lower maternal food intake of the treated animals. No macroscopic abnormalities of organ or skeletal structure were seen in fetuses from Group II. The litters in Groups I and III gained weight normally until weaning, and no differences in gait (a neurotoxic effect) were observed. No abnormalities were found in the brains, spinal cords or sciatic nerves of these rats at 6 weeks of age, either macroscopically or by light microscopy in this relatively insensitive (too few animals) study. Edwards (1976) also showed that acrylamide was present in fetal tissues at levels similar to that of dams. These findings have been corroborated in other species by other investigators using ^{14}C-labeled acrylamide (Ikeda et al., 1983, 1985; Marlowe et al., 1986).

Walden et al. (1981) monitored the concentrations of selected intestinal enzymes in rats exposed to acrylamide (20 mg/kg/day) in utero and during lactation. While significant changes in the levels of acids and alkaline phosphatases, beta-glucuronidase, citrate synthetase, and lactate dehydrogenase were observed, a consistent pattern of toxicity did not emerge, and the toxicological importance of these findings is not apparent.

Zenick et al. (1986) exposed female Long-Evans rats to acrylamide in drinking water at levels of 0, 25, 50 or 100 ppm for 10 weeks. Exposure began two weeks prior to mating and continued throughout mating, gestation, and lactation, Hindlimb splaying appeared in the 100 ppm group during the first to second

week of pregnancy. Body weight and fluid intake were also depressed. Dams in the 50 ppm group showed depression of these parameters during the last two weeks of lactation. Acrylamide did not significantly affect mating performance, pregnancy rates, litter size or survival. However, acrylamide did significantly depress pup body weight at birth (100 ppm group) and weight gain during lactation through post-weaning, day 42 (50 and 100 ppm groups). Vaginal patency was delayed in the 100 ppm group only. These data indicate that acrylamide has a deleterious effect on pup development when dams are exposed at levels of 50 ppm and above, suggesting that 25 ppm is a NOEL for this endpoint.

In a modified one-generation reproduction study, groups of 20 female Sprague-Dawley rats were treated with 0, 25, or 50 ppm of dietary acrylamide for 2 weeks before mating (Biodynamics, 1979; Spencer and Schaumberg, 1977). After mating, the rats were maintained on the 25 or 50 ppm acrylamide diets during 19 days of gestation. Although the rats on the 50 ppm of dietary acrylamide gained less weight, there were no statistically significant effects due to acrylamide treatment on maternal body weight, maternal mortality, mating or pregnancy rate. The pups did not display any gross malformations at birth and there were no statistically significant differences in litter size, pup weight or viability between controls and groups exposed to acrylamide. At weaning (day 21 of age), 4 pups from the control group (2 of each sex) and 8 pups from each of the treatment groups (4 of each sex) were examined histologically for neurotoxicity. Four of the

pups exposed to acrylamide in utero (dose unspecified) displayed Wallerian degeneration in the tibial nerve, compared with one of the pups from the control group. Three of the pups exposed to acrylamide in utero (dose unspecified) had unilateral optic nerve degeneration; no optic nerve degeneration was observed in the control group. There was some fiber degeneration in the sciatic and optic nerves of the treated, but not the control pups.

Thus, it appears that in utero exposure to acrylamide results in neurotoxic manifestations in the offspring, possibly at a dose which produced no overt signs in the mother. Since these neurotoxic manifestations are degenerative in nature, they cannot be considered to be an adverse effect on the development of the conceptus. Rather, it appears that these effects occurred during late gestation (after major organogenesis) or in the post-natal, pre-weaning period.

The available pharmacokinetics data suggest that the half-life of acrylamide metabolites is up to 8 days (see section 4). Since dosing of the dams continued until gestation day 19, and acrylamide is known to cross the placenta (Ikeda et al., 1983, 1985; Marlowe et al., 1986), it is expected that significant quantities of acrylamide or its metabolites could reach the late-gestation fetus as well as the neonate during the first few post-natal days. These considerations could provide an explanation for the presence of degenerative changes in the neonate following exposure of the dam during gestation.

The apparent discrepancy between these results (fetal effects at 25 or 50 ppm) and those reported by Edwards (1976; little or no direct fetal effects at 400 ppm) is striking. The major protocol difference is that American Cyanamid Company (1980) dosed animals for 2 weeks prior to mating, whereas Edwards (1976) began oral dosing from gestation day 1. Dosing for the 2 additional weeks may have resulted in more acrylamide or its metabolites being available via the milk during the pre-weaning period, thereby raising acrylamide concentrations in the neonate to toxic levels.

Zenick et al. (1986) show that effects in the neonate were observed at dose levels similar to those used by American Cyanamid Company (1980). At the high dose, maternal toxicity was clearly more severe in the Zenick study than in the American Cyanamid study. While the nature and extent of the neonatal effects reported in Zenick et al. suggest that they result from maternal toxicity, the neurotoxic effects reported in American Cyanamid suggest a primary effect on the fetus. Although the American Cyanamid study is an incomplete protocol and is not fully reported, this apparent discrepancy in results should not be ignored. Strain-specificity in rats, observed in acrylamide-induced male reproductive toxicity (see section 6.3), may be a possible explanation. Further study is needed before definite conclusions can be reached.

6.3. Male Reproductive Effects

Ali et al. (1983) studied the effect of acrylamide on the levels of circulating testosterone, growth hormone, and prolactin in male F344 rats. Animals were injected ip, daily for 20 days, with an aqueous solution of acrylamide at doses of 0, 10 or 20 mg/kg. Twenty-four hours after the last treatment, the animals were decapitated and hormones were assayed by radioimmunoassay. A dose-dependent depression of testosterone and prolactin concentrations was observed, with statistical significance seen only at the 20 mg/kg dose ($P<0.05$). Growth hormone concentration was not altered. Decreased levels of testosterone may lead to decreased testicular function resulting in impotence, reduced seminal volume, and sterility.

The toxic effect of acrylamide and several related compounds on the testes was investigated by Hashimoto et al. (1981). Male mice (ddY strain, 5 to 6 weeks of age, weighing about 29 g) were distributed randomly into groups of 5 to 7 animals. Groups were dosed orally with test compounds, twice weekly for 8 to 10 weeks, at a concentration of 35.5 mg/kg. In order to examine the effect of metabolic activation, a parallel study was conducted in which mice were injected ip with phenobarbital (PB) at a dose of 50 mg/kg for 5 successive days per week, from 1 week prior to the study start until the last week of the study.

Acrylamide was neurotoxic at 35.5 mg/kg, producing ataxia and weakness in the hindlimbs. It also produced both testicular atrophy and significant reduction in testes weight, with

degeneration of the epithelial cells of the seminiferous tubules. However, the interstitial cells were normal. Testicular damage appeared to be completely prevented or reduced by PB pretreatment, as demonstrated in the parallel study. The authors concluded that PB may enhance the inactivation of acrylamide. Since methacrylamide (another neurotoxic compound) did not produce any testicular effects when given at a neurotoxic dose, the data suggest that neurotoxicity and testicular toxicity may occur by different mechanisms.

In a subsequent study, Sakamoto and Hashimoto (1986) demonstrated that acrylamide decreased the fertility rate in male ddY mice. Increased resorptions per dam resulted when treated male or female mice were mated to unexposed animals. These effects on reproduction occurred at levels above the threshold for neurotoxicity. The dosing regimen was significantly different from that used in the earlier study. Animals were given acrylamide in the drinking water 4 to 6 weeks prior to mating. In the earlier report, twice weekly oral intubations were given for 8 to 10 weeks. While pharmacodynamics and other factors must be evaluated before a firm conclusion can be reached, it appears that the reproductive organs are not more sensitive to acrylamide toxicity than is the peripheral nervous system.

The reproductive effects of acrylamide also have been evaluated in a dominant lethal/two-generation reproduction assay. Acrylamide was administered to male and female F344 rats in the

drinking water for 10 weeks (Nalco Chemical Co., 1987). Dose levels were 0, 0.5, 2.0 and 5.0 mg/kg/day. Following mating of these treated males to the treated females, the treated males were remated to untreated females (mating periods unspecified). Following mating, administration to treated females continued throughout gestation and lactation. The same basic protocol was followed for the second parental generation except that treatment was for 11 weeks.

The dominant lethal results are as follows:

1. Although the number of eggs ovulated per untreated dam was equal among all dose groups, there was a significant reduction in total implants per litter at 5.0 mg/kg/day. Therefore pre-implantation loss was significantly increased.

2. There was also a significant increase in non-viable implants per litter, i.e. resorptions, at 5.0 mg/kg/day. Accordingly, post-implantation loss was increased.

The reproductive results (first generation) are as follows:

1. Although males given acrylamide were able to successfully mate with females (no effect on mating index), the litter size and the number of litters was reduced at 5.0 mg/kg/day compared to controls. Since both sexes were dosed, it is not clear whether this is a male- or female-mediated observation. Although the similarity of these results with those observed in the dominant lethal assay suggest that this is a male-mediated effect, this does not preclude the possibility of an effect in females as well.

2. Additionally, parental animals in the 2.0 and 5.0 mg/kg/day groups displayed evidence of ataxia. Animals in these groups had lower body weight gains than the controls.

The results of the second generation are as follows:

1. No effect on mating, number of females pregnant, or gestation length.

2. At 5.0 mg/kg/day the number of implantations per dam and the number of live pups per litter were significantly reduced. Post-implantation losses were significantly increased.

3. Gestation weight gain was decreased at 2.0 and 5.0 mg/kg/day. Lactation weight gain was increased at 5.0 ug/kg/day.

Acrylamide-induced peripheral neuropathy was produced in male rats at 5.0 mg/kg/day. Thus, it appears that acrylamide is reproductively toxic in the rat at doses which may also be neurotoxic.

Shelby et al. (1986) administered acrylamide either as a single ip dose at 125 mg/kg or as 5 daily doses at 50 mg/kg/day to hybrid mice (C3H x 101) in a dominant lethal assay. Subsequent matings to T-stock and C3H x 101 females showed significant post-implantation loss following both dosing regimens, from matings between days 4.5 and 11.5 after treatment. This study suggested that late spermatids and early spermatozoa were affected.

Zenick et al. (1986) exposed male Long-Evans rats to acrylamide in drinking water at levels of 0, 50, 100 or 200 ppm (0, 4.2, 7.9, and 11.6 mg/kg/day, respectively) for 10 weeks. In

males, copulatory behavior, seminal parameters, fertility (controls and 100 ppm only) and fetal outcomes were evaluated using untreated females. Hindlimb splaying was apparent in the 200 ppm group by week 4 and less severe splaying appeared in the 100 ppm group at week 8. Disruptions in copulatory behavior preceded the appearance of this ataxia. These disruptions in mating performance interfered with ejaculatory processes and subsequent transport of sperm, since semen was found in the uterus of only one of the females mated with the 100 ppm group at week 9. Moreover, only 33% of the females mated (week 10) to the 100 ppm group were pregnant. Post-implantation loss was also significantly increased. These data indicate that acrylamide had a deleterious effect on copulatory behavior, fertility, and fetal survival. As fertility and fetal outcomes were not evaluated at 50 ppm, the NOEL in the male could not be determined. Since effects were dependent upon cumulative dosing (effects at 1/2 dose level took twice as long to be observed) it is possible that neurological effects were occurring, but not yet evident, at these low levels (see section 5).

To determine the coexistence of neurological and reproductive effects, a male reproductive study was conducted at lower drinking water doses (0, 15, 30 and 60 ppm or 0, 1.5, 2.8, and 5.8 mg/kg/day, respectively) with Long-Evans rats (Smith et al., 1986). Males were exposed to acrylamide for a total of 80 days. After 72 days, treated males were mated with untreated females until each had impregnated two females or until day 80.

Females were sacrificed on day 14 of gestation and examined for corpora lutea and for living and dead fetal implants. Fertility rate and pre- and post-implantation losses were measured. In addition, half of the males were sacrificed and cytological preparations of the testes and the sacral, sciatic,and tibial nerves were made. The remaining males were sacrificed 12 weeks later.

There were no effects on body weight, food and fluid intake, or fertility. Pre- and post-implantation losses were both evident at the high dose (60 ppm), while post-implantation loss was also evident at the mid-dose (30 ppm). No gross or histopathological signs of nerve damage were observed at any dose. Upon sacrifice immediately following treatment, testicular preparations showed no increase in aberrations. In animals sacrificed 12 weeks later, a total of 4 reciprocal translocations were observed in the treated animals (1, 1, and 2 in the 15, 30, and 60 ppm groups, respectively).

In a subsequent study using higher doses, Sublet et al. (1986) observed effects at times that suggest that spermatozoa and spermatids represent the germ cell stages most sensitive to acrylamide's effects. These investigators have shown that the reproductive toxicity of acrylamide in the Long-Evans rat is a primary effect on testicular tissue and its contents, since the effects were observed in the absence of neurotoxicity. These findings are strengthened by the results of Marlowe et al. (1986) who studied the distribution of ^{14}C-labeled acrylamide in male

Swiss-Webster mice by whole-body autoradiography. They observed that significant amounts of acrylamide appeared in the testes one hour after oral administration. Radioactivity was present up to 9 days following injection, indicating that acrylamide has ample opportunity to exert a direct toxic effect on the testes.

McCollister et al. (1964) exposed male Dow-Wister rats (number unspecified) to 800 ppm of acrylamide in the diet for 10 days, followed by 400 ppm for 9 days. Following 51 days of recovery, gross and microscopic examination revealed marked tubular degeneration in the testes of the two remaining rats (the others had died during and after dosing). In addition, histological examination revealed no effect on the testes at 30 ppm. The testes of intermediate dose animals (70, 90, 110 and 300 ppm) apparently were not examined histologically, so a threshold dose has not been defined. These data provide limited information on reproductive toxicity, as testicular effects were noted only at lethal doses.

A sperm-head abnormality study was conducted in B6C3F1 mice exposed to acrylamide by 5 daily doses at 100 mg/kg ip (J. Meier, USEPA, Cincinnati, OH, personal communication). Increased incidences of sperm-head abnormalities and decreased testes weight to body weight ratios were observed.

7. GENOTOXIC EFFECTS

7.1. Summary and Conclusions

For years, the neurotoxicity of acrylamide has been the primary basis for its human health concern. Consequently, it appears that neither the mutagenic nor the carcinogenic potential of this important industrial chemical has been extensively examined until recently. Despite this relatively late interest in acrylamide's potential mutagenicity and carcinogenicity, evidence for acrylamide's genotoxic potential has appeared in the literature.

The major concern for acrylamide's genotoxicity centers on its clastogenic activity. This effect appears more pronounced in the germ cells as compared to somatic cells. The interaction with germinal tissues suggests the possible heritability of acrylamide-induced DNA alterations. If the transmission of acrylamide-altered DNA is demonstrated, this could have implications for a human health concern for future generations. Other reports support the evidence that acrylamide can interact with DNA, e.g., DNA binding and induction of aneuploidy and polyploidy. On the other hand, the weight of evidence from available reports suggests acrylamide may not produce detectable gene mutations. Since most of this evidence is derived from *in vivo* studies, it is not clear at this point whether or not acrylamide requires metabolic activation to exert its genotoxic effects. The genotoxicity results available to EPA to date are summarized in Table 7.1

The revised guidelines for mutagenicity risk assessment published by EPA (51 FR 34006, 1986) allow for an evaluation of the potential genetic risk associated with human exposure to acrylamide. The genotoxicity data for acrylamide suggest an intrinsic mutagenic (clastogenic) activity and provide sufficient evidence for chemical interaction in the mammalian gonad. The body of evidence suggests that acrylamide may induce alterations in the genome of germinal cells. These data provide a fairly strong weight of evidence bearing on the potential for human germ-cell mutagenicity and its heritability. Heritable translocation results increase the strength of a human germ-cell mutagenicity concern to a level that would only be further strengthened by direct human evidence (the highest level of evidence for human mutagenicity). Data from a rigorous, well-conducted heritable translocation assay may allow for a possible quantitative risk assessment that may be tied to human exposure data concerning acrylamide.

Table 7-1 Summary Table of Genotoxic Results for Acrylamide

Assay	Result[a)]	Highest Dose Tested	Reference
Gene Mutations			
Salmonella strains TA97,TA98,TA100, TA102,TA1535,TA1537, TA1538 ± activation	negative	30 mg/plate	Lijinsky & Andrews, 1980 American Cyanamid, 1983a Bull et al., 1984a Institut d'Hygiene et d'Epidemiologie, 1985 Hashimoto & Tanii, 1985 NTP, 1985 EPA, unpublished results
Mouse Lymphoma - no activation	positive	850 ug/ml	Moore et al., in press
CHO/HPRT, ± activation	negative	1200 ug/ml	American Cyanamid, 1983b
Drosophila sex-linked recessive lethal - feeding	negative	100 ppm	American Cyanamid, 1985a
Chromosomal effects - in vivo			
Aberrations in mouse bone marrow - feeding (in diet)	negative	500 ppm	Shiraishi, 1978
- i.p. injection	negative	100 mg/kg	Shiraishi, 1978
Aberrations in mouse spermatogonia - feeding (in diet)	positive	500 ppm	Shiraishi, 1978
- i.p. injection	positive	100 mg/kg	Shiraishi, 1978

Table 7-1 Summary Table of Genotoxic Results for Acrylamide (continued)

Chromosomal effects - in vivo	Result[a)]	Highest Dose Tested	Reference
Dominant lethal			
in rats - drinking water	positive	5.8 mg/kg/day	Smith et al., 1986
in mice - single or multiple i.p. injections	positive	125 mg/kg 5x50 mg/kg/day	Nalco Chemical Co., 1987 Shelby et al., 1986
Mouse micronucleus - oral gavage	negative	2x75 mg/kg/day	American Cyanamid, 1983c
- oral gavage	negative	5x100 mg/kg/day	EPA, unpublished results
Heritable translocations			
in mice - multiple	positive	5x40 mg/kg/day	Shelby et al., 1987
i.p. injections	positive	5x50 mg/kg/day	Shelby et al., 1987
Chromosomal effects - in vitro			
Human blood lymphocytes			
- aberrations	positive	50 ug/ml	Institut d'Hygiene et
- SCE	negative	50 ug/ml	d'Epidemiologie, 1985
CHO/SCE - 2 hr. exposure, ± activation	positive	3.25 mg/ml	EPA, unpublished results
24 hr. exposure, no activation	positive	0.175 mg/ml	EPA, unpublished results
Micronucleus - Chinese hamster fibroblasts - 3 hr exposure	negative	10^{-3} M	Zaichkina and Ganassi, 1984
Mouse Lymphoma - no activation	positive	850 ug/ml	Moore et al., in press

Table 7-1 Summary Table of Genotoxic Results for Acrylamide (continued)

Chromosomal effects - in vivo	Result[a)]	Highest Dose Tested	Reference
Other effects			
Mouse spermhead abnormality - oral gavage	weak positive	5x100 mg/kg/day	EPA, unpublished results
UDS/primary rat hepatocytes	positive	3.3 mg/ml	American Cyanamid, 1983e, 1985b
	negative	10^{-2} M	Miller and McQueen, 1986
Transformation with: C3H/10T 1/2, no activation	positive	200 ug/ml	Banerjee and Segal, 1986
NIH/3T3 cells, no activation	positive	200 ug/ml	Banerjee and Segal, 1986
BALB/c-3T3 cells, + activation	positive	800 ug/ml	American Cyanamid, 1985c, d
Mitotic recombination in *Saccharomyces* - D_7, ± activation	negative	500 ug/ml	Institut d'Hygiene et d'Epidemiologie, 1985
Amplication of SV40 DNA inserts of SV40 transformed Chinese hamster cells	weak positive	150 ug/ml	Vanhorick and Moens, 1983
Alteration of transfection of *E. coli* CR63 cells	positive	10 ug/0.1 ml	Vasavada and Padavatty, 1981

a) results are determined for all testing conditions listed

7.2. Gene Mutation Assays

7.2.1. Salmonella Assays

Acrylamide has been tested for mutagenicity by several investigators in the Salmonella/mammalian activation assay. The evidence indicates acrylamide does not induce gene mutations in this assay (Lijinsky and Andrews, 1980; American Cyanamid Co., 1983a; Bull et al., 1984a; Institut d'Hygiene et d'Epidemiologie, 1985; Hashimoto and Tanii, 1985; NTP 1985). Acrylamide did not produce Salmonella revertants in strains TA98, TA100, TA1535, TA1537, and TA1538 with or without activation. In some instances, concentrations up to 30 mg/plate were used and both plate incorporation and liquid suspension procedures were performed.

Furthermore, acrylamide was tested in the plate incorporation method with the newer Salmonella strains TA97 and TA102 (J. Meier, USEPA, Cincinnati, OH, personal communication). The results were negative up to a dose of 30 mg/plate, with or without activation.

7.2.2. Eukaryotic Gene Mutation Assays

Acrylamide was tested for its ability to induce mutations at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus in cultured Chinese hamster ovary (CHO) cells at doses up to 1200 ug/ml with and without metabolic activation (American Cyanamid Co., 1983b). Although increased mutation frequencies were

observed at 300 ug/ml with activation and at 37.5 ug/ml without activation, a dose-response relationship was not observed. However, because these increases appear at unique doses, an additional experiment should be performed to confirm these results before suggesting potential mutagenic activity by acrylamide in this assay.

Acrylamide was tested without exogenous activation in mouse lymphoma L5178Y TK+/- cells for mutation at the thymidine kinase locus (Moore et al., 1987). Acrylamide induced a dose-dependent increase in mutation frequency up to concentrations of 850 ug/ml. All doses gave survivals above 10%. It appears that the increased mutation frequency is due almost exclusively to induction of small colonies. Moore et al., (1985) have suggested that the mouse lymphoma assay may be capable of evaluating not only gene mutations, but clastogenic events as well. Small colony formation appears to represent chromosomal alterations whereas large colonies represent gene mutations (Hozier et al., 1985). These data with mouse lymphoma cells, therefore, may represent a clastogenic event (see Section 7.3.2).

A Drosophila sex-linked recessive lethal assay was performed by feeding 100 ppm acrylamide to Canton-S wild type males for 72 hours (American Cyanamid Co., 1985a). These were mated to Basc stock females for a total of three broods. The dose of 100 ppm caused 100% sterility in broods 2 and 3. Therefore, 100 ppm acrylamide was fed to males for 24 hours. Sterility was still noticeable, but the assay was able to be performed. Over 5,000

lethal tests each were examined in the treated and the control groups over two experiments. No increase in the percent lethals was observed over controls indicating that acrylamide did not induce sex-linked recessive lethals in post-meiotic germ cells of treated males.

7.2.3. Summary of Gene Mutation Assay Results

Acrylamide does not appear to induce gene mutation in three types of gene mutation assays examined: the Salmonella/mammalian activation assay, the CHO/HPRT mutation assay, and the Drosophila sex-linked recessive lethal assay. The mouse lymphoma mutation data suggest that acrylamide may induce mutations in an eukaryotic gene mutation assay, but these data may reflect a clastogenic event due to the predominant formation of small colonies. It may be relevant that the CHO sub-clone generally used in the CHO/HPRT mutation assay does not appear to be sensitive to clastogens (Hsie et al., 1986). The remaining assay that would confirm the apparent lack of gene mutation activity by acrylamide, as well as possible heritability resulting from such activity, would be the mouse specific locus assay. However, due to the weight of evidence indicating no mutational activity, this assay is not recommended for further assessment regarding gene mutations.

7.3. Chromosomal Assays

7.3.1. In Vivo Effects

The first evidence demonstrating that acrylamide induced chromosomal effects was reported by Shiraishi (1978). Acrylamide was administered to male mice either 1) in the diet (500 ppm) for 1, 2 or 3 weeks exposure, or 2) by a single ip injection of 50, 100, 150 or 200 mg/kg with animals held for 12 and 24 hours and 11 and 12 days post-exposure (LD_{50} determined to be 200 mg/kg; only doses of $\leq$100 mg/kg provided data). No increase in frequency of chromosome aberrations was observed in bone marrow under any conditions. However, significant increases in chromosome breaks (up to 19% vs. 2.4% controls) and clear chromatid exchanges (up to 6% vs. 0% controls) were observed in spermatogonia over 2 to 3 weeks of feeding. Increases in breaks were also observed 11 to 12 days post-injection (up to 12.5% vs. 3% controls). There were, however, noticeable decreases in mitotic activity in both bone marrow and spermatogonia. The total number of aneuploid and polyploid cells increased with time after treatment in both these cell types. Acrylamide also induced significant increases in aberration frequency in primary spermatocytes under both treatment conditions. The aberrations included univalents, fragments such as breaks and deletions, and rearrangements such as chain quadrivalents and ring quadrivalents. Sister chromatid exchange (SCE) frequencies in bone marrow and spermatogonia were not increased over controls under

any treatment condition. Acrylamide significantly reduced testes weight suggesting possible cell killing. These results suggest a genotoxic hazard to the male germ cell posed by acrylamide. This report is noteworthy as it demonstrates that a chemical is clastogenic to germ cells, but not to the examined somatic cells in the same test animal.

Further evidence for germ cell genotoxicity in the absence of cell atresia, predominantly through chromosomal effects, is found in several dominant lethal studies in rodents. Smith et al. (1986) exposed male Long-Evans rats to 15, 30 and 60 ppm (0, 1.5, 2.8 and 5.8 mg/kg/day, respectively) acrylamide in their drinking water. They were treated for ten weeks and then mated at the end of this period. There were no observable differences in weight gain or fluid consumption from controls and the fertility rate was comparable in all dose groups. An increased pre-implantation loss was seen only at the highest dose where an approximately 2.5-fold elevation over the negative control was observed. An increased post-implantation loss was observed at the two highest doses with up to a six-fold increase over the negative control. Also, no peripheral neuropathies as determined by light microscopy of the sciatic nerve nor hindlimb splaying (characteristic of acrylamide neuropathy) were observed.

These investigators also performed a preliminary dominant lethal study using a 5-day treatment regimen followed by serial matings (Sublet et al., 1986). Rats were dosed with 0, 5, 15, 30, 45 and 60 mg/kg/day (100 mg/kg/day was lethal) and then mated

at 1, 2, 3, 4, 7 and 10 weeks after exposure. Significant pre-implantation losses were observed above 5 mg/kg and post-implantation losses at doses greater than 15 mg/kg. The results from these two studies appear to suggest that acrylamide affects the spermatid-spermatozoa stages.

The Smith et al. (1986) study also examined cytogenetic endpoints. After breeding the male rats for the dominant lethal study, one-half of the males in each group were sacrificed and testicular preparations were made. The remaining males were sacrificed 12 weeks later for analysis of possible reciprocal translocations in spermatocytes derived from treated spermatogonial stem cells. There was no increase of aberrations in animals at the first sacrifice. From preliminary analyses, they have noted the presence of 4 reciprocal translocations found in treated males and none in controls at 12 weeks. Although no statistics were applied, these investigators concluded that this is suggestive, but not conclusive, evidence of an acrylamide effect.

Smith et al. (1986) reported negative findings, based on preliminary observations, for chromosome aberrations in differentiating spermatogonia at high, acute doses of acrylamide. One dose of 125 mg/kg (ip) was given to six C3H x 101 mice and spermatogonial cell preparations were made. No chromosomal aberrations were observed in a total of 100 metaphase cells each from the control and treatment groups.

Shelby et al. (1986) reported positive results in a mouse dominant lethal study after acrylamide exposure. Male (C3H x 101) F_1 hybrid mice were exposed with either a single 125 mg/kg ip dose (maximum tolerated dose) or 5 multiple 50 mg/kg/day ip doses of acrylamide. Males were mated with either T stock females or (SEC x C57BL) F_1 hybrid females. An observed increased frequency of dead implants was apparent from matings 4.5 and 11.5 days after treatment, with the multiple treatment more effective than the single dose. These results were seen at times that would suggest an acrylamide-induced effect on the spermatid-spermatozoa stages.

Another dominant lethal study was performed with F344 rats (Nalco Chemical Co., 1987). Male rats were exposed to acrylamide in drinking water at doses of 0, 0.5, 2.0 and 5.0 mg/kg/day for 10 weeks prior to breeding. A dominant lethal effect was seen at the top dose only. Mating performance was unaffected although animals had lower body weight gains compared to controls. At 5 mg/kg, the number of corpora lutea per dam were unaffected, the number of implants per litter were significantly reduced causing a significant increase in pre-implantation loss compared to controls (24.9% vs. 14.3% control), and the number of viable implants per litter were significantly reduced, causing a significant increase in post-implantation loss compared to controls (14.3% vs. 6.2% control). These studies provide evidence that acrylamide can reach the germ cells via an <u>in vivo</u> exposure and produce a genotoxic effect.

Confirmation of the ability of acrylamide to induce heritable germ cell mutagenic effects comes from a study of treated male mice. Shelby et al. (1987) administered acrylamide intro-peritoneally (either 40 mg/kg/d or 50 mg/kg/d for 5d), mated them and screened male progeny for heritable translocations by noting reductions in fertility. A number of presumptive carriers of translocations were confirmed by cytogenetic analysis. A highly significant increase in translocations was noted in the male progeny, being 39%, 24% and 0.2% in the 50 mg/kg/d, 40 mg/kg/d and historical control groups, respectively. In summary, the dominant lethal studies in rats and mice and the mouse heritable translocation study demonstrate the ability of acrylamide to produce transmissible germ cell mutagenic effects.

Acrylamide has been tested in two studies to assay its ability to induce chromosome breakage in mouse bone marrow cells as measured by an increase in micronuclei. In the first study (American Cyanamid Co., 1983c), male and female CD-1 mice were exposed to acrylamide by gavage under two regimens. Mice were exposed to either 1) 75 mg/kg and sacrificed and cells harvested 30 and 48 hours later, or 2) 2 x 75 mg/kg (24 hours apart) and sacrificed and cells harvested 48 and 72 hours from initial treatment. The 75 mg/kg dose produced piloerection, hypersensitivity and abnormal gait. Lethality was seen at doses of 100 mg/kg and above. One thousand polychromatic erythrocytes were scored from each of eight animals (4 of each sex). Although there were small, absolute increases in micronuclei numbers with

either treatment regime, these increases did not appear biologically significant.

Another mouse micronucleus study also examined the effect of acrylamide on CD-1 mice (J. Meier, USEPA, Cincinnati, OH, personal communication). Ten animals (5 of each sex) were exposed to 5 consecutive daily doses of 25, 50 or 100 mg/kg/day by gavage. Bone marrows were harvested 6 hours after the last dose. The negative results corroborate the ones from the previous study. However, there did not appear to be any gross signs of toxicity in contrast to many reports at the 100 mg/kg/day dose. These micronucleus results are consistent with the Shiraishi (1978) study where no chromosomal aberrations were found in bone marrow.

7.3.2. In Vitro Effects

Acrylamide has been tested for chromosomal effects in cultured mammalian cells. Acrylamide was examined for its ability to induce chromosome aberrations and sister chromatid exchanges (SCE) in peripheral blood lymphocytes from human donors (Institut d'Hygiene et d'Epidemiologie, 1985). Phytohemmaglutinin-stimulated lymphocytes were exposed to acrylamide at concentrations of 0.1 to 50 ug/ml for 72 hours. Acrylamide induced significant increases in aberrations (breaks, fragments, dicentrics, rings and minute chromosomes were scored). The frequency of SCE/cell was slightly enhanced over baseline, but neither a doubling at any dose nor a dose-response was

observed. Acrylamide also increased the incidence of aneuploidy and polyploidy. The observed effects were independent of blood donor.

The clastogenic response to acrylamide was examined in mouse lymphoma cells in culture (Moore et al., in press). Acrylamide induced both chromatid and chromosome breaks and rearrangements. A clear dose-response for clastogenicity was observed at concentrations up to 850 ug/ml (64 aberrations/100 cells) without metabolic activation. These results appear to support the hypothesis discussed earlier (see section 7.2.2) that mouse lymphoma cells may be capable of detecting clastogenic and mutagenic events by discrimination of colony size after mutant expression.

Acrylamide was examined for its ability to induce SCE in CHO cells in culture. CHO cells were exposed to acrylamide for either 24 hours (doses up to 0.175 mg/ml) or 2 hours (doses up to 3.25 mg/ml) (J. Meier, USEPA, Cincinnati, OH, personal communication). There was a significant increase in SCE/cell, but no doubling of control values, after the 24-hour exposure without activation (tests with microsomal activation were not conducted). For the 2-hour treatment, a significant doubling of control values was seen with or without activation. The level of induced SCE/cell under activated conditions was not greater than that seen under nonactivated conditions. This suggests a possible direct acting mechanism for acrylamide. In another SCE study (American Cyanamid Co., 1983d), the reported results were

negative, but the negative control values were exceptionally high. While the effects at all doses were similar to the baseline values, this study should be repeated with acceptable negative control values to be considered valid.

A report, translated from Russian, suggests that acrylamide does not induce micronuclei in cultured Chinese hamster fibroblasts (Zaichkina and Ganassi, 1984). Acrylamide was tested at only one concentration (10^{-3}M) for a 3-hour exposure and the cells were apparently fixed after a 24-hour culture period. However, this study is too limited to reach definitive conclusions.

7.3.3. Summary of Chromosomal Assay Results

The studies examining the chromosomal effects of acrylamide indicate its clastogenic potential. This effect appears to be more pronounced in the germ cells than in somatic cells. The suggestive reciprocal translocation results raise the possibility of acrylamide-induced alterations to DNA that may be transmissible to future generations. Indeed, acrylamide is currently being tested in a mouse heritable translocation assay to test for its possible heritable effects (Shelby and Generoso, personal communication).

In vivo as well as the *in vitro* results suggest acrylamide may induce aneuploidy. Aneuploidy and polyploidy are consistent effects in studies which scored for these endpoints. However, the reviewers in the Aneuploidy Program sponsored by the U.S. EPA

suggest that this does not permit the distinction of an exclusive aneuploidy-inducing ability by acrylamide as the aneuploidy and polyploidy numbers were reported as one figure (Cimino et al. 1986). Acrylamide should be tested for its ability to produce aneuploidy using appropriate tests and measurements.

Since most of the clastogenic activity was noted in in vivo studies, it is uncertain whether metabolic activation is required to exert its genotoxic effects. The in vitro results suggest a possible direct acting mechanism for acrylamide. The CHO/SCE study found increases in SCE/cell under nonactivated conditions, albeit at relatively high concentrations. In vitro studies with human lymphocytes in which clastogenicity was observed were performed without activation. However, the metabolic activating capability of lymphocytes has not been totally explored and it is known that lymphocytes are capable of activating chemicals such as cyclophosphamide to genotoxic forms (Waalkens et al., 1981). The mouse lymphoma results demonstrate that acrylamide induces aberrations without activation. However, this direct acting mechanism may not be entirely clarified by these results. These mouse lymphoma cells have been found to detect the mutagenicity of 2-acetylaminofluorene without addition of exogenous exzymes (M. Moore, USEPA, Research Triangle Park, NC, personal communication). Since 2-acetylaminofluorene is believed to require metabolic activation to exert its genotoxic effect, these cells may retain residual exogenous metabolic capability.

Whether this capability is class specific or not has not been addressed.

7.4 Other Genotoxic Effects

There are several reports indicating that acrylamide is capable of binding to DNA (see Section 4). Briefly, it has been shown that radiolabeled acrylamide binds to protein and nucleic acids in rodents (Hashimoto and Aldridge, 1970; Carlson and Weaver, 1985). Furthermore, there is evidence to suggest that acrylamide is capable of direct DNA alkylation via Michael addition (Solomon et al. 1985). These investigators noted that acrylamide reacted most strongly with adenine in *in vitro* reactions with calf thymus DNA.

Two studies were performed to evaluate the effect of acrylamide on mouse sperm-head morphology (J. Meier, USEPA, Cincinnati, OH, personal communication). In both studies B6C3F1 mice were exposed to acrylamide at 0, 25, 50 and 100 mg/kg/day for 5 days by gavage. Significant mortality was found at the top dose. Animals were sacrificed at 3 weeks and 5 weeks. There was a significant drop in the testes weight: body weight ratio at the upper doses. However, the number of sperm per mg of cauda epididymis remained similar between control and treated groups. At 3 weeks, significant increases in the number of abnormal sperm-heads at 50 and 100 mg/kg/day were noted, including banana, blunt hook, amorphous, pinhead and two headed morphologies. There was a large increase in abnormal spermheads at the 100

mg/kg/day dose, but it is not clear whether this may be due to cytotoxicity or genetic effects at this relatively high dose. At 5 weeks, a response was apparent, but increased numbers of abnormal sperm seen at the top dose only. Again, whether or not this is due to cytotoxicity is uncertain.

There are conflicting reports regarding the ability of acrylamide to induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. Acrylamide was tested in two UDS assays by American Cyanamid Company (1983e, 1985b). It induced a dose-dependent increase in UDS up to 600 ug/ml (American Cyanamid Co., 1985b). However, only the top dose produced an increase greater than 5 net nuclear grains (9.1 $\pm$ 0.9 with triplicate cultures) with 88% of the cells in repair ($\geq$4 net nuclear grains). Cytotoxicity was seen at doses of 750 ug/ml and higher. In the other UDS assay (American Cyanamid Co., 1983e), higher doses were apparently able to be assayed and large increases in UDS were observed (e.g. >40 net nuclear grains at doses of 1 and 3.3 mg/ml). Doses of 10 mg/ml and higher appeared cytotoxic. Another report suggests that acrylamide is negative in a similarly performed assay (Miller and McQueen, 1986). Concentrations of acrylamide up to 10^{-2} M induced no apparent increase in DNA repair. This concentration is similar to the doses tested in the previous reports, 600 to 1,000 ug/ml. Higher concentrations in this study were cytotoxic to the hepatocytes. In support of the negative finding, density gradient studies confirmed the lack of induced DNA repair as [^{3}H] thymidine was

not found to incorporate into nonreplicative DNA after acrylamide exposure (Miller and McQueen, 1986). These same investigators further report that acrylamide did not interfere with the repair of uv light-induced DNA damage. The conflicting UDS data preclude an unequivocal determination of the effect of acrylamide on DNA damage and repair. It should be noted that the variability of the UDS hepatocyte test is affected by several factors including the functional state of the animals and the isolated cells (Lonati-Galligani et al., 1983).

Acrylamide has been tested in several in vitro transformation assays. It exhibited dose-dependent transformation in both NIH/3T3 mouse fibroblast cells and C3H/10T 1/2 cells up to 100 and 150 ug/ml, respectively (Banerjie and Segal, 1986). All morphologically transformed cells were subsequently shown to grow in soft agar, which suggests malignant potential by the transformed cells. Two additional in vitro transformation assays with BABL/c-3T3 cells examined under activated conditions support the published finding (American Cyanamid Company, 1985c, 1985d). These two assays examined acrylamide's transforming potential over two dose ranges (10 to 100 ug/ml and 100 to 800 ug/ml) in the presence of exogenous metabolic activation. Acrylamide apparently produced little cytotoxicity, but induced a dose-dependent increase in morphological transformants (e.g., type III foci) within each study.

Acrylamide was not able to significantly increase the mitotic recombination frequency at the trp 5 locus in Saccharomyces cerevisiae D_7 (Institut d'Hygiene et d'Epidemiologie, 1985). Saccharomyces were exposed to acrylamide concentrations of 1 to 500 ug/ml with or without exogenous activation, for either 2 hours with shaking at 37°C or 17 hours with shaking at 29°C. The top dose was slightly toxic to the yeast, but the conversion frequency was not increased with either treatment.

Acrylamide is a weak inducer of the amplification of SV40 DNA inserts of SV40 transformed Chinese hamster cells (Vanhorick and Moens, 1983). This effect might result from a weak DNA-damaging activity demonstrated by the ability of high concentrations of acrylamide to inhibit the DNA synthesis rate of the cells. Furthermore, acrylamide synergistically increased the enhancement of SV40 DNA amplification by 6 established carcinogens in a dose-dependent manner. This potentiation of effects by other chemicals may need to be taken into account when assessing the risk of acrylamide. Amplification of SV40 DNA may provide intrachromosomal sites for DNA breakage (DNA-damaging activity). The polycopy of the SV40 inserts of the infected cells is a suggested model for replicon amplification induced by carcinogens. The authors suggest this amplification may be a molecular mechanism for carcinogenesis initiation.

Acrylamide was tested for its ability to alter the transfection of E. coli CR63 cells with E. coli B lysate of

colitis bacteriophages (Vasavada and Padayatty, 1981). Transfection by phage DNA is inhibited by alteration(s) to the transfecting DNA (e.g., mutation, DNA cleavage). Acrylamide-treated phage DNA (10 ug acrylamide in 0.1 ml H_2O) produced a 50% inhibition of transfection ability as compared to non-treated phage control. Acrylamide does not appear to interfere with the transfection process as transfection of E. coli CR63 cells pre-treated with acrylamide was not reduced when transfected with non-treated phage DNA. The authors suggest acrylamide is interacting with phage DNA and producing alterations that change the transfection efficiency.

Evidence presented in this section on other genotoxic effects suggests that acrylamide is capable of interacting with DNA and producing a biological consequence. The transformation results and possibly the amplification of SV40 inserts suggests acrylamide produces effects that may have implications for its carcinogenic potential.

8. CARCINOGENIC EFFECTS

8.1. Summary and Conclusions

Administration of acrylamide in the drinking water to F344 rats for 2 years caused a statistically significant increase in the incidence of tumors (benign and/or malignant) in dosed animals at the following tumor sites: CNS uterus, mammary and thyroid gland (females); scrotum, thyroid, and adrenal gland (males). Furthermore, in a series of one year limited bioassays in mice, acrylamide was shown to: (1) be a skin tumor initiator in SENCAR mice (gavage, i.p., and dermal) and Swiss ICR mice (gavage); and (2) induce lung adenomas in strain A/J mice (oral and i.p. routes), Swiss ICR mice (gavage, and SENCAR mice (i.p.).

Additional support for the conclusion that acrylamide is a carcinogenic agent is provided by acrylamide's genotoxicity. Acrylamide has been shown to be a clastogenic agent both *in vivo* and *in vitro*. Preliminary reports suggest that acrylamide binds to DNA, induces DNA damage and repair effects, and causes *in vitro* cell transformation. Acrylamide also has been shown to cause dominant lethal effects.

OTS has concluded that there is a "sufficient" weight of evidence from long-term studies in animals and from additional supporting studies to identify acrylamide as a "Group B2" carcinogen -- probable carcinogen in humans, as defined in EPA's Chemical Carcinogen Risk Assessment Guidelines. Although the chemical has only been studied in one long-term bioassay in rats, it induced statistically significant increased incidences of

tumors in multiple tissues in both sexes. Further, several of the tumor incidences were dose-dependent. Additional evidence for the conclusion is provided by positive data from a series of limited bioassays in mice, from a series of genotoxicity studies, and from in vivo and in vitro DNA binding studies.

The International Agency for Research on Cancer (IARC) has reviewed the evidence regarding the carcinogenic potential of acrylamide, and has concluded that acrylamide is a Group 2B carcinogen--sufficient evidence for carcinogenicity in animals, with no data or inadequate data on humans available (IARC, 1986).

8.2. Epidemiology Studies

American Cyanamid studied the relationship of worker exposure to acrylamide and cancer mortality at its Warners Plant (Collins, 1984). The investigation by American Cyanamid was based on analyses of two study groups: a long duration exposure cohort (10 individuals) and a short duration/intermittent exposure cohort (52 individuals). A standardized proportionate mortality ratio (SPMR) was utilized to analyze the data because information was not available for the entire population at risk. Results from the study indicated no significant excesses of all types of cancer mortality had occurred among the exposed employees. Although mortality from cancers of the lung and central nervous system appeared to be slightly elevated, these findings were based on small numbers of cases and the SPMRs were not significantly different from expected values. Many factors

limited the interpretation of the study. These limitations included: underrepresentation of the employee population potentially at risk for exposure-related effects, incomplete ascertainment of causes of death for cohort members, incomplete acrylamide exposure data, and the small size of the cohort (Pickrel et al., 1986).

Because of the limitations noted, the Warners Plant cohort study does not provide an adequate assessment of the mortality experience of workers exposed to acrylamide. The investigation by Collins (1984), admittedly, was limited only to information abstracted from available company records. As a result of this restriction, the cohort may have been incomplete and biased with respect to employee acrylamide exposure and cancer mortality. The analysis of two separate exposure cohorts may have also precluded a proper interpretation of workers' cancer mortality patterns. Analyses of combined data for two cohorts indicated that significant excesses of lung cancer mortality may have occurred (Obs/Exp = 8/4.64, SPMR = 1.72, p = 0.6). However, the relatively small cohort size and low level of statistical power associated with the study precluded further interpretation of these results.

In a study by Sobel et al. (1986), the mortality experience of 371 employees assigned to acrylamide monomer and polyacrylamide operations was examined. Emphasis was given to those cancer sites identified from animal studies (see Johnson et al., 1986); i.e., tumors of the central nervous system, thyroid

gland, other endocrine glands, and mesotheliomas. Whereas 38 deaths were expected, a total of 29 deaths was observed up until 1982. No deaths were classified for the sites identified from the animal studies. However, 11 deaths due to malignancies were noted versus 7.9 expected (an SMR of 139). When those employees with previous dye exposure were excluded, the obs/exp ratio changed to 4/6.5 for an SMR of 61. In Collins (1984) a slight excess of lung and central nervous system deaths was observed. While an excess of deaths from respiratory system cancers was seen in Sobel et al., obs/exp, 4/1.9, SMR 202, a deficit resulted when employees with previous dye exposure were removed (obs/exp, 1/2.4).

American Cyanamid followed up this work with a more detailed historical prospective cohort mortality investigation, similar to that of Sobel et al. (1986). It was a more involved analytic follow-up to the small proportionate mortality ratio analysis of Collins (1984). The study was performed at three U.S. plant locations - Warners, Fortier, and Kalamazoo - and at the Botlek facility in the Netherlands. Employees were engaged in different jobs with potential exposure to either or both production of acrylamide monomer or its polymerization into polyacrylamide.

The maximum number of years for the worker(s) with the longest duration of exposure contrasts with the "average" number of years of duration at the plants. These figures, along with the estimates of numbers of departments, job descriptions and exposed workers are listed below in Table 2. The table helps to

show that the great majority of person-years of exposure occurred at the Warners plant, where the average duration of exposure is higher only than that at the Botlek plant. It also shows that, although all the plants produced acrylamide, the industrial hygienists have categorized many more jobs as "exposed" at Warners. It is possible some of these jobs had minimal exposure, which, if true, would dilute the comparisons.

Table 2. Estimates of Exposed Jobs, Titles, Workers, and Years of Exposure

Plant	Departments	Job Titles	Exposed Workers	Years of Exposure Maximum	Years of Exposure Average
Warners	9	70	1355	30	6.3
Fortier	7	8	634	18	7.2
Kalamazoo	5	11	37	15	10.3
Botlek	9	31	267	18	5.7

There was a statistically significant excess of total cancer for the combined Warners cohort (448 obs / 412.6 exp / SMR 109/ p = 0.04), a difference of 35.4 cases. No information was given that might have contributed to understanding this excess. When the entire cohort was separated into exposed and unexposed subcohorts, there was an excess of 35.6 total cancer cases in the unexposed group. It may be due to another occupational exposure.

Twenty-five cancer sites were examined, but because of the low number of expected deaths this much analysis need not have been performed. Just considering the SMRs among the exposed subcohorts combined from the four plants, excesses appeared for

cancer of the pancreas (8 obs / 4 exp / SMR 200), respiratory tract (30 obs / 26.3 exp / SMR 114), and lung cancer (30 obs / 25.1 exp / SMR 120); none were statistically significant ($p > 0.15$). There were no notable deficits in observed deaths.

Limitations of this study included: the inexplicable inclusion of 1,533 exposed workers in the "unexposed" group; inadequate exposure data concerning acrylamide, and no data concerning other exposures; no specification of the standard population used to compute expected numbers of deaths in the trend analysis, and no analysis of time from initial exposure to death from cancer.

It is difficult to accept this report as evidence of no association between acrylamide exposure and cancer excess without further information clarifying the apparent inclusion of 1,533 exposed workers in both the unexposed and exposed groups. Especially troubling is the idea that deaths among these exposed workers may have been tabulated in the unexposed totals.

The expected rates in the trend analysis for the unexposed are not the same as those the authors used earlier in the study, apparently because a different, internal, standard was used. Because the comparison population has not been specified, the validity of this analysis cannot be ascertained. If these "unexposed" workers - who show a statistically significant excess of respiratory cancer, compared with U.S. white male rates (169 obs / 128.6 exp / SMR 1.31 / $p = 0.0004$) - have been exposed to

other potentially carcinogenic agents, this internal comparison would not be a suitable one.

Acrylamide exposures at the four plants began as early as 1954, but no monitoring was conducted until 1977. The Warners New Products department was never monitored. Thus, the approaches to estimating exposures do not constitute an exposure assessment.

Overall, the results of these three studies do not indicate a statistically significant excess of cancers from exposure to acrylamide, although all three studies showed apparent increases in lung or respiratory cancers among acrylamide-exposed workers, and lung cancer incidence was increased in the mouse oncogenicity work (Bull et al., 1984a,b). Small sample sizes in Collins (1984) and Sobel et al. (1986), the ambiguous definitions of the study groups in Collins et al. (1987), and the incomplete exposure assessments in all three studies were insufficient, however, to accurately assess any causal role for acrylamide related to cancer incidence. Consequently, all of the studies discussed here are inadequate to judge the human carcinogenicity of acrylamide.

8.3. Two-Year Bioassay Data

A chronic bioassay on acrylamide has been performed by Dow Chemical Company (Johnson et al., 1984, 1986).

8.3.1. Summary of Protocol/Conduct of Study

Several dose levels were administered via the drinking water to both sexes of F344 rats: 0.0, 0.01, 0.1, 0.5, and 2.0 mg/kg body weight/day for two years. The purity of the test material ranged from 98.1% upwards, with soluble polymer (230 to 600 ppm) being the major contaminant. The concentration of acrylamide after four days in the drinking water in the cage bottles was at least 92% of the original concentration, therefore drinking water solutions were prepared twice a week. The amount of acrylamide in the drinking water was within acceptable variance of the targeted level of all dose levels as determined by high performance liquid chromatography (HPLC) on a C_{18} column, and the dosing method is considered to have been adequate. Ninety rats/sex/dose were started on the study, but only 60 rats/sex/dose were part of the group designated for the 2-year terminal sacrifice. The remainder of the rats were used for either interim sacrifices at 6, 12, or 18 months, or a separate electron microscopy assessment of the nervous system. (See neurotoxicity section.)

Parameters evaluated were mortality, clinical signs of toxicity, body weight, food consumption, water consumption, clinical chemistry, hematology, urinalysis, organ weight, gross pathology, and histopathology.

8.3.2. Limitations of Study

No major design or conduct limitations are apparent in the Dow study. There were transient symptoms in some rats consistent

with a viral infection (sialodacryoadenitis virus) that occurred beginning on day 210 of the study. All animal groups (both males and females, including controls) were equally affected. The symptoms are judged not to significantly confound the results of the carcinogenicity study. The viral infection has been shown not to significantly affect body weight, survival, and incidences of adrenal tumors, mammary tumors and leukemia of F344 rats (Rao et al. 1988).

8.3.3. Reported Results

The MTD appears to have been achieved at the high-dose level based on decreased survival, decreased body weight gain, and the observance of several toxic effects at the high-dose. Mean body weights of high-dose rats were less than controls - male rats at the high-dose reached a maximum difference of about 4% less than controls after a year or more on test. Males given 0.5 mg/kg/day and females given 2.0 mg/kg/day had mean body weights about 2% less than their respective control groups. Increased mortality was observed in high-dose rats, especially females. The increase occurred only beginning at about the 21st month when mortality in males at the high-dose was 6/60 compared to 3/60 in controls, and mortality in females was 11/60 compared to 4/60 in controls. At termination, both males and females in the high-dose group showed statistically significant increases in mortality: males 25/60 and females 32/60 compared to male and female control mortality of 16/60 and 10/60, respectively. Also histopathology revealed

that female high-dose rats showed degeneration of the peripheral nerve (tibial) and some degeneration of the spinal cord; recognized neurotoxic effects of acrylamide.

According to the study authors, increased incidences of a variety of tumors (benign and/or malignant) were statistically significant ($p \leq 0.05$) at the high-dose, including; females - mammary gland (benign and malignant), CNS (malignant), thyroid gland - follicular epithelium (combined benign and malignant), mouth (benign), uterus (malignant), clitoral gland (benign) and pituitary gland (benign); males-scrotal mesothelioma (malignant), thyroid gland follicular epithelium (benign), and adrenal gland-pheochromocytoma (benign). An EPA analysis of selected tumor sites revealed that the incidence of benign pituitary gland tumors (females) was not statistically significant. Note that both treated males and females showed a statistically significant increased incidence of tumors derived from the follicular epithelium of the thyroid gland. Tumor incidence data for the above-mentioned sites are summarized in Table 8.1. Pooled tumor incidence data are summarized in Table 8.2.

a. Testes Scrotal Cavity

The only tumor type that was significantly increased in males at a dose below the high-dose was scrotal mesothelioma in rats given 0.5 mg/kg/day. The incidence of scrotal mesothelioma does not appear to be significantly increased in rats given 0.01 or 0.1 mg/kg/day, although the incidence of scrotal mesothelioma

Table 8.1. Tumor Incidence Data from the Lifetime Drinking Water Bioassay

Tumor Site/Type	Males: Number with tumor/number at risk[d]/dose[e] (statistical significance)					Females: Number with tumor/number at risk[d]/dose[e] (statistical significance)				
	Controls	0.01	0.1	0.5	2.0	Controls	0.01	0.1	0.5	2.0
1) Testes-Scrotal Cavity										
Mesothelioma	3/57	0/50	7/57	11/53(a)	10/54(a,b)	----	----	----	----	----
2) Thyroid Gland										
Adenoma (AO	1/57	0/53	2/57	1/53	7/54(a,b)	0/54	0/55	1/50	1/54	3/50(b)
Adenocarcinoma(AC)	----	----	----	----	----	1/54	0/55	0/50	0/54	3/50
A,AC, or Follicular Tumor	----	----	----	----	----	1/54	0/55	1/50	1/54	5/50(b)
3) Mammary Gland										
Adenocarcinoma	----	----	----	----	----	2/58	1/58	1/52	2/55	6/57(c)
Total Benign Adenoma	----	----	----	----	----	10/60	11/60	9/60	19/58	23/60(a)
Combined Benign and Malignant						10/60	12/60	10/60	20/58(b)	28/60(g)
4) CNS										
Combined Glial Tumors or "proliferations"[f]	5/60	2/60	0/60	3/60	8/60	1/60	2/59	1/60	1/60	9/60(a)
Glial tumors (without "proliferations")	5/60	2/60	0/60	2/60	6/60	1/60	2/59	1/60	0/60	7/60(g)
5) Adrenal Gland Pheochromocytoma										
Benign	3/57	8/50	7/57	5/52	10/54(a,b)	----	----	----	----	----
Malignant	2/57	0/50	2/57	1/52	0/54					
Combined	5/57	8/50	9/57	6/52	10/54(NS)					

Table 8.1. Continued

Tumor Site/Type	Males: Number with tumor/number at risk[d]/dose[e] (statistical significance)					Females: Number with tumor/number at risk[d]/dose[e] (statistical significance)				
	Controls	0.01	0.1	0.5	2.0	Controls	0.01	0.1	0.5	2.0
6) Clitoral Gland										
Adenoma	----	----	---	----	----	0/2	1/3	3/4	2/4	5/5(a,b)
Adenomacarcinoma	----	----	---	----	----	0/2	0/3	0/4	2/4	0/5
Combined						0/2	1/3	3/4	4/4	5/5
7) Uterus Adenocarcinoma	----	----	---	----	----	1/56	2/56	1/51	0/55	5/49(a)
8) Oral Cavity										
Squamous Papilloma	4/57	7/50	0/57	5/53	4/54(NS)	0/58	3/58	2/52	1/56	7/56(a,b
Squamous Carcinoma	2/59	0/53	1/58	0/58	2/56(NS)	0/60	0/60	0/60	2/60	1/60
Combined	----	----	----	----		0/60	3/60	2/60	3/60	8/60(a,b
9) Pituitary Gland	----	----	----	----	----	25/59	30/60	32/60	27/60	32/60(a)(f
Adenoma	----	----	----	----	----	7/59	9/60	1/60	3/60	5/60
Adenocarcinoma	----	----	----	----	----	32/59	39/60	33/60	29/60	37/60(NS)
Combined										

a = Dow designated as statistically significant, Fisher Exact probability test, =0.05
b = Dow designated as statistically significant, mortality adjustment via Mantel-Haenszel procedure (Deto) =0.05
c = Dow designated as linear trend by Mantel-Haenszel extension of Cochran-Armitage test =0.05
d = number at risk is the number of rats assumed to be alive at time of appearance of the first tumor
e = dose in mg/kg/day in drinking water
f = includes glial proliferations that are suggestive of an early tumor
g = $P \leq 0.05$ by Fisher Exact probability test
NS = Not significant by Fisher Exact probability test

Table 8-2. Pooled Tumor Incidence Data

	Dose (mg/kg/day)				
Males	0.0	0.01	0.1	0.5	2.0
Number of Animals with Tumors[a] (testes, thyroid adrenal[b])	7/57	8/53	13/57	14/53	22/54
Number of Animals with Malignant Tumors (testes)	3/57	0/53	7/57	11/53	10/54
Females					
Number of Animals with Tumors[a] (thyroid, mammary, CNS[c], oral, uterus)	13/60	18/60	14/60	21/60	46/60
Number of Animals with Malignant Tumors (thyroid, mammary, CNS, uterus)	4/60	5/60	3/60	4/60	20/60

[a] = Benign or malignant. Site must be statistically significant at the high-dose (treated vs. control) for tumors to be considered.
[b] = includes only adrenal benign tumors
[c] = tumors only (no "proliferations")

exceeded concurrent and historical control mean values in male rats given 0.1 mg/kg/day. Note that although concurrent controls in the Dow study had an incidence of scrotal mesothelioma of 5.3%, Dow states in its report that the historical incidence is 3.8% for this tumor type in control males at 24 months, and the NTP lists mesothelioma as 1.3%, and 1.0%, for the testes and peritoneal cavity, respectively, for F344 rats. The percentage incidence of scrotal mesothelioma in the present Dow study was (dose in mg/kg/day): 5.3% (0.0), 0% (0.01), 12.3% (0.1), 20.8% (0.5), and 18.5% (2.0).

b. Thyroid

The only tumors for which both sexes showed a statistically significantly increased incidence were those derived from the follicular epithelium of the thyroid gland. Both males and females showed a statistically significant increase in the incidence of thyroid adenoma at the high-dose. Further, adenocarcinomas were found in three females in the high-dose group.

c. Mammary Gland

The incidence of benign adenoma is statistically significant in females at the high-dose, and the increased incidence of combined adenoma and adenocarcinoma is significant at both the high-dose (P=0.00047) and the next lowest dose level (P=0.022). There appears to be a time-to-tumor (latency) effect for both adenocarcinoma and total benign tumors of the mammary gland; this possible latency effect was assessed for adenocarcinoma from the

individual animal data EPA received. Although the occurrence of adenocarcinoma was not (according to Dow) statistically significant by either pairwise analyses used by Dow, 5 of the 6 adenocarcinomas seen in high-dose females were observed before termination whereas the 2 adenocarcinomas in control females were not observed until termination of the bioassay (day 743). The five high-dose females with adenocarcinoma were evaluated on day 577 (spontaneous death), 593 (moribund), 677 (moribund), 680 (spontaneous death), and 732 (moribund). The cause of death of 2 of these 5 females was attributed by Dow to the mammary tumors, 1 to pituitary tumor compression of the brain, and undetermined cause of death for the other 2 females. This apparent decreased tumor latency supports the statistically significant trend seen for adenocarcinoma.

d. Clitoral Gland

There appears to be a dose-dependent response in combined benign and malignant tumors of the clitoral gland, and there is a statistically significant increase in adenomas (benign) and in combined benign and malignant tumors in females at the high-dose. Due to the low number of clitoral glands examined (see Table 8.1), this tumor site was not included in the pooled tumor incidence data in Table 8.2. However, it is important to note that even with such small numbers of tissues examined, a rather high incidence of tumors was observed. Historically, clitoral adenoma occurs in F344 female rats of the NTP testing program at an average rate of 1.2% (Haseman et al., 1984), and Dow's

historical control incidence of clitoral adenomas in F344 rats is listed in their report as 0.5%.

e. Oral Cavity

Tumors originating from the mucosa of the mouth are shown in Table 8.3. Females show a statistically significant increase in papillomas, and combined papillomas and carcinomas, at the high-dose when tumors of the "tongue" are combined with those of "oral tissues". The Dow historical control incidence in female F344 rats for squamous cell papilloma of the oral cavity is 2.2% (range 0-4%). Additionally, according to the Dow report, an increased incidence of focal cell hyperplasia of the hard palate was observed in both males and females, and the incidence of this hyperplasia was statistically significantly increased in males at both 0.5 and 2.0 mg/kg/day.

f. Uterus

Adenocarcinoma of the uterus was significantly increased in high-dose females compared to controls. Endometrial stromal polyps were also reported in this study; however, their occurrence may not be related to acrylamide exposure because they are commonly found in 344 rats.

g. Nervous System

Table 8.4 shows the combined incidence of glial tumors (malignant) or "proliferations" in the CNS. Glial proliferations are not considered by Dow to be tumors, but rather to be

Table 8-3. Tumors Originating from the Mucosa of the Mouth[a]

	Dose (mg/kg/day)				
Female Rats	0.0	0.01	0.1	0.5	2.0
Squamous papillomas, benign, origin from tongue, hard palate or lip	0/58	3/58	2/52	1/56	7/56(b,c)
Squamous cell carcinoma, malignant, origin from hard palate or gingiva	0/60	1/60	0/60	2/60	1/60
Combined papilloma and carcinoma	0/60	3/60	2/60	3/60	8/60(b,c)
Male Rats					
Squamous papillomas, benign, origin from tongue, hard palate or lip	4/57	7/50	0/57	5/53	4/54
Squamous cell carcinoma, malignant, origin from tongue, hard palate, gingiva or pharynx	2/59	0/53	1/58	0/58	2/56
Combined papilloma and carcinoma	6/59	7/53	1/58	5/58	6/56

a = Papillomas and carcinomas occurred in separate rats, according to the Dow authors

b = Dow designated as statistically significant, Fishers' exact probability test, = 0.05

c = Dow designated as statistically significant, mortality adjustment via Mantel - Haenszel procedure (Peto), = 0.05

Table 8-4. Combined Incidence of Glial Tumors of the CNS.

MALES	Dose (mg/kg/day)				
Brain	0.0	0.01	0.1	0.5	2.
Astrocytoma	3/60	0/60	0/60	2/60	2/60
Glial Proliferation (suggestive of early tumor)	0/57	0/50	0/57	1/53	1/54
Oligodendroglioma	0/60	2/60	0/60	0/60	1/60
Spinal Cord (Cervical, Thoracic, and Lumbosacral)					
Astrocytoma	1/59	0/53	0/58	0/58	3/56
Undifferentiated glial cell tumor	1/44	0/47	0/46	0/44	0/35
Glial proliferation	0/44	0/47	0/46	0/44	1/35
Total rats with a tumor of glial origin	5/60	2/60	0/60	2/60	6/60
Total rats with a tumor of glial origin or a glial proliferation suggestive of early tumor	5/60	2/60	0/60	3/60	8/60
FEMALES					
Brain					
Astrocytoma	0/58	1/58	0/52	0/56	3/
Glial proliferation (suggestive of tumor)	0/56	0/56	0/51	1/55	3/
Oligodendroglioma	0/56	1/56	1/51	0/55	1/49
Spinal Cord (Cervical, Thoracic, and Lumbosacral)					
Astrocytoma	1/60	0/59	0/60	0/60	3/60
Total rats with a tumor of glial origin	1/60	2/59	1/60	0/60	7/60[c]
Total rats with a tumor of glial origin or a glial proliferation suggestive of early tumor	1/60	2/59	1/60	1/60	9[a]/60[b]

a = one female rat given 2.0 mg/kg/day had an astrocytoma in the cervical section of the spinal cord and glial proliferation in the brain.

b = Statistically significant increase over control according to Dow (no details on level of significance).

c = $P \leq 0.032$.

suggestive of early astrocytomas. However, one consulting veterinary pathologist for Dow considered these lesions to be astrocytomas. When brain and spinal cord are considered separately, these individual categories do not appear to be statistically significant for either sex. However, when combined, the high-dose females have an increased incidence of total glial tumors and proliferations. Note the glial proliferations in females only occurred in the two highest dose groups. Combined CNS glial malignant tumors (omitting glial proliferations) are also statistically significantly increased in females at the high-dose (P=0.032). Additionally, other combinations of glial malignant tumors were cited by Dow to be statistically significant (i.e., astrocytomas from brain and cord, or astrocytomas and glial proliferations from brain and cord) for females. However, neither the incidence data or the results of the statistical analyses were presented. In males, the increased number of CNS tumors or proliferations at the high-dose was not statistically significant. However, it should be noted that the concurrent control incidence (8.3%) was greater than either Dow's historical controls (1.0% or NTP's historical controls (<1%)) for F344 male rats.

h. Adrenal Gland

A statistically significant increase in benign pheochromocytoma of the adrenal medulla was observed in high-dose males. A significant increase in foci (multifocal) of

altered cells in the adrenal cortex was observed at the 0.1 and 2.0 dose levels, although an increasing dose-response trend was not observed. This foci increase may indicate a preneoplastic condition and as such supports the contention that this tumor type may be chemically induced. Note that neither malignant, nor combined (p=0.11) tumor incidence was statistically significantly elevated in males.

i. Pituitary Gland

According to the Dow report, a statistically significant increase in the incidence of benign tumors of the pituitary gland was observed in high-dose females, but this difference was not significant according to a statistical analysis (Fisher Exact test) performed by EED/EPA.

The incidence of pituitary adenoma, and adenocarcinoma, in Dow concurrent controls was 42.4%, and 11.9%, respectively; in Dow historical controls 35.7%, and 12.4%; and in NTP historical controls 44.0%, and 3.5%, for these tumor types. Thus, the incidence of adenomas in Dow concurrent controls (42.4%) is somewhat higher than in Dow historical controls (35.7%). Also, it should be noted that the NTP historical control incidence for adenocarcinoma (3.5%) is much less than the Dow concurrent controls (11.9%) and Dow historical controls (12.4%). The high incidence of pituitary tumors in the Dow concurrent controls may be due to the influence of the viral infection which occurred beginning on day 210 of the study. Rao et al. (1988) have shown

that male F344 rats with sialodacryoadenitis virus infection have a significantly higher incidence of pituitary tumors. Additional support for the biological significance of the pituitary adenomas is the fact that adenomas in the high-dose females were generally noted earlier, and were larger, than those in the control group. However, the treated animals do not show an increase in adenocarcinoma, and this is of course a factor in the combined tumor incidence, which is also not statistically significant.

In summary, there is some weak support for the biological significance of pituitary tumors in high-dose females. However, a statistical analysis of pituitary tumors by EED/EPA has revealed a lack of statistical significance for pituitary tumor incidence in females ($P<0.16$ for adenomas), thus pituitary tumors are not included in the pooled tumor incidence in Table 8.2.

8.4. Second Lifetime Oncogenicity Study in Rats with Acrylamide -- (American Cyanamid Company, Final Report, June 27, 1989)

A second lifetime oncogenicity study in rats with acrylamide was designed to clarify some ambiguities (considered by American Cyanamid Company) of the prior carcinogenicity study by Johnson et al., (Toxicol. Appl. Pharmacol. 85:154-168, 1984).

8.4.1. Background and Summary of Results

Statistically significantly increased incidences of mammary gland tumors (fibroadenomas, or fibroadenomas and adenocarcinomas combined), scrotal mesotheliomas, and thyroid neoplasms (adenoma, or adenoma and carcinoma combined, in both males and females)

were observed in Fischer 344 rats administered acrylamide in the drinking water. Increased incidences of uterine adenocarcinomas, clitoral gland adenomas, and papillomas of the oral cavity observed in the prior study were not found in the rats of this study. However, there was a slight increase in cutaneous fibromas in the female high-dose group of this study. Furthermore, there was a dose-related positive trend in the incidence of malignant reticulosis of the brain in the dosed female rats and the incidence of CNS glial tumors (astrocytomas) was higher in the high-dosed male and female rats than in controls. The totality of findings in this study confirm the multiple-sites carcinogenicity of acrylamide in the rat.

8.4.2. Summary of Protocol/Conduct of Study

Groups of male and female Fischer 344 rats (6-7 weeks of age) were administered acrylamide (99.9% pure) in their drinking water for 2 years (106-108 weeks) at doses of: 0.1, 0.5 or 2.0 mg/kg/day (male); 1.0 and 3.0 mg/kg/day (female). The body weight range of male rats at the start of the study was 72-144 g while that of the female rats was 75-122 g. In the male rat study, two independent control groups of 102 male rats in each group were used; the low-, mid-, and high-dose groups consisted of 204, 102 and 75 animals, respectively. In the female rat study, two independent control groups were also used consisting of 50 female rats in each group; the low- and high-dose groups each consisted of 100 animals.

Mortality, clinical signs of toxicity, body weight, food consumption, and water consumption were measured during the study. A complete necropsy was done on all animals sacrificed at the termination of the study and on those animals which were sacrificed in a moribund condition or that died during the study. At terminal sacrifice, weights of brain, liver, kidneys and testes were obtained. Microscopically, the thyroid and the testes were examined in all male rats. In addition, the brain, spinal cord and gross lesions were examined in all control and high-dose male rats and in all low- and mid-dose rats found dead or sacrificed moribund.

8.4.3. Limitations of Study

No major design or conduct limitations are apparent in this study. The MTD appears to have been achieved at the high-dose level based on decreased body weight gain, decreased survival, and the observance of several toxic effects at the high-dose.

8.4.4. Reported Results

The mean body weight of the high-dose (2 mg/kg/day) male group was consistently lower than that of each individual control group or the combined control groups with maximum decrease (8.6%) at the end of the study. There were no differences in the mean body weight between the other treated male groups and the control groups. The mean body weight of the high-dose (3 mg/kg/day)

female rats was significantly decreased compared to controls as early as week 3 of the study and reached a maximum decrease of 9.2% at week 92. The mean body weight of the low dose (1 mg/kg/day) female rats was decreased from that of the controls early in the study but from week 32 until the end of the study differences from controls were insignificant.

There was increased mortality in the high-dose male group (2 mg/kg/day) beginning from month 17 and continuing until the end of the study. At the end of the study, mortality was 75% in this group compared to 58.5% in the control groups. In the females, increased mortality was observed in the high-dose group during the last month of the study. Total mortality reached 49% at the end of the study compared to 34% mortality in the control groups. There was no increased mortality in the low- or mid-dose male or the low-dose female groups. At terminal sacrifice, there were slight differences in organ weight which were occasionally statistically significant. There were increased incidences of the palpable masses and mild degeneration of the peripheral nerve in the high-dose group of both sexes.

Under the conditions of this study, there were increased incidences of CNS glial tumors (astrocytoma) and of thyroid follicular cell neoplasms of both males and females at the high-dose of 2 and 3 mg/kg/day, respectively. In addition, there was a significant increased incidence of scrotal mesotheliomas at the high-dose males and of mammary gland neoplasms in both low- and high-dose females (1 and 3 mg/kg/day) when compared to that of

the control groups. Although not statistically significant, there was a dose-related positive trend in the incidence of malignant reticulosis of the brain in the dosed female rats and there was a slight increase in cutaneous fibromas in the female high-dose group. Increased incidences of uterine adenocarcinomas, clitoral gland adenomas, and papillomas of the oral cavity observed in the prior study were not found in this study. The tumor incidence data for each organ site are summarized in Tables 1-4.

a. Testes Scrotal Cavity

The incidence (13/75, 17.3%) of mesotheliomas of the testes was statistically significantly ($P<0.001$) increased in the high-dose (2.0 mg/kg/day) male group in comparison to the control group (4/102, 3.9%). Furthermore, there was a slight, but not statistically significant, increased incidence (8/102, 7.8%) in the mid-dose group (0.5 mg/kg/day) (Table 1). Of the 13 high-dose rats diagnosed with scrotal mesothelioma, only 2 were identified at the terminal sacrifice. The other 11 were identified in animals found dead or sacrificed moribund, with the earliest dead occurring in week 66 of the study, and a total of 6 identified prior to week 91. The short tumor latency is indicative of the carcinogenic potency of acrylamide.

b. Thyroid

There was a slight increase in the incidences of follicular cell carcinomas in both the high-dose male and female groups, which were not statistically significant. However, both male and female rats in the high-dose groups (2 and 3 mg/kg/day, respectively), as well as females of the low-dose group (1 mg/kg/day), had statistically significantly increased incidences of thyroid follicular cell adenoma, or adenoma and carcinoma combined. Thyroid follicular cell adenomas were seen in 2/100 (2%) and 1/102 (1%) controls, 9/203 (4.4%) low-dose, 4/101 (4%) mid-dose, and 15/75 (20%) high-dose male rats; the incidences in the controls (two groups), low- and high-dose females were 0/50 (0%), 0/50 (0%), 7/100 (7%), and 16/100 (16%), respectively. Combined thyroid follicular cell adenomas and carcinomas were seen in 3/100 (3%) and 3/102 (3%) control, 12/203 (5.9%) low-dose, 4/102 (4%) mid-dose, and 17/75 (22.7%) high-dose males; the incidences in female rats were: 1/50 (2%) and 1/50 (2%) controls, 10/100 (10%) low-dose, and 23 /100 (23%) high-dose (Table 2).

c. Mammary Gland

The incidences of mammary fibroadenomas, or combined fibroadenomas and fibrocarcinomas were statistically increased in both acrylamide treated female groups (1 and 3 mg/kg/day) as compared with those in the control groups. Mammary fibroadenomas were seen in 5/46 (10%) and 4/50 (8%) controls, 20/94 (20%) low-

dose, and 26/95 (26%) high-dose female rats; the incidences of fibroadenomas and fibrocarcinomas (combined) in the female rats were: 7/46 (15%) and 4/50 (8%) controls, 21/94 (22%) low dose, and 30/95 (32%) high dose. There was no significant difference in the incidence of hyperplasia or adenocarcinoma between the treated rats and the controls (Table 3).

d. Brain and Nervous System

There were no significant differences in the incidence of combined brain and spinal cord (CNS) total glial tumors (astrocytomas and oligodendrogliomas) between control and treated rats, male or female. However, the incidence of astrocytoma, a CNS tumor of glial origin, was statistically significantly increased ($P<0.01$) in the male high-dose groups (3/75, or 4%) as compared with that in the controls (1/102, or 1% and 0/102, or 0%); the incidence of astrocytomas in high-dose females was also higher (3/100, or 3% vs. 0/100, or 0% in controls). Furthermore, an infrequently occurring non-glial tumor of the brain, malignant reticulosis, was diagnosed in 2 low-dose and 3 high-dose female rats; 1 control male and 1 low-dose male but no female control rats had this neoplasm (Table 4). The numbers of brain and spinal cord (CNS) sections examined histopathologically in this study were not specified; in the prior study, greater numbers of sections of these tissues were examined (brain -- 6 sites vs. 3 routinely; spinal cord -- 3 sites vs. 1).

e. Skin

There was a slight increase in cutaneous mesenchymal neoplasms (fibromas) in the female high-dose group (3 mg/kg/day) with a total of 5 fibromas in the high-dose group versus 1 in the combined control groups.

A variety of cutaneous epithelial neoplasms were observed in all groups of animals in this study. The incidence of these neoplasms was similar in all groups.

f. Other tissues

In this study, there were no significant increased incidences of tumors of the oral cavity, clitoral gland, or uterus in the dose groups.

8.5. Limited Bioassays (One-Year Studies)

Bull et al. (1984, a,b) showed that acrylamide acted as a skin tumor initiator in both SENCAR mice and Swiss-ICR mice. Acrylamide also increased lung adenoma incidence in SENCAR, Swiss-ICR, and A/J strains of mice (Bull et al. 1984a, b; Robinson et al 1986).

Female SENCAR mice (6-8 weeks old, 40 mice per dose level) were administered acrylamide at 0,12.5, 25.0 or 50.0 mg/kg/application of acrylamide for a total of 6 applications over a 2-week period by gavage, i.p., and dermal routes. Two weeks after the final dose of acrylamide was applied (representing the initiation phase of the experiment), 1.0 ug of

TABLE 1

Incidence of Scrotal Mesotheliomas

Group	1	2	3	4	5
Dose (mg/kg/day)	0	0	0.1	0.5	2.0
No. Examined	102	102	204	102	75
Animals with Mesothelioma, Testes tunic	4	4	9	8	13*
Per cent	3.9	3.9	4.4	7.8	17.3

*Significantly different from combined controls, $p<0.001$ (Peto, 1980, and Tarone, 1975)

TABLE 2

Incidence of Thyroid Follicular Cell Lesions

	Males					Females			
Groups	1	2	3	4	5	1	2	3	4
Dose (mg/kg/day)	0	0	0.1	0.5	2.0	0	0	1.0	3.0
No. in Group	102	102	204	102	75	50	50	100	100
No. Examined	100	102	203	101	75	50	50	100	100
Adenoma	2	1	9	4	15*	0	0	7*	16*
%	2	1	4.4	4	20*	0	0	7*	16*
Carcinoma	1	2	3	0	3	1	1	3	7
%	1	2	1.5	0	4	2	2	3	7
Hyperplasia	1	2	7	3	2	0	1	5	1
%	1	2	3.4	3	2.7	0	2	5	1
Combined	3	3	12	4	17**	1	1	10*	23**
Neoplasms%	3	3	5.9	4	22.7**	2	2	10*	23**

*Fisher exact, $P<0.05$
*Statistically significant, $p<0.001$ (Peto, et al., 1980)

TABLE 3

Incidence of Primary Mammary Gland Neoplasms

	Females			
Group	1	2	3	4
Dose (mg/kg/day)	0	0	1.0	3.0
No. in Group	50	50	100	100
No. Examined	46	50	94	95
Fibroadenoma	5	4	20*	26*
%	10	8	20*	26*
Adenocarcinoma	2	0	2	4
%	4	0	2	4
Combined	7	4	21*	30*
%	15	8	22*	32*

*Fisher's Exact, $p<0.05$
**Statistically significant, $p<0.001$, (Peto, et al., 1980)

TABLE 4

Incidence of Primary CNS Glial Tumors

	Males					Females			
Groups	1	2	3	4	5	1	2	3	4
Dose (mg/kg/day)	0	0	0.1	0.5	2.0	0	0	1.0	3.0
No. Examined	102	102	98	50	75	50 50	50 50	100 100	100 100
Astrocytoma	1	0	1	0	3*	0	0	2	3
%	1	0	1	0	4*	0	0	2	3
Oligodendro-glioma	0	1	1	1	0	0	0	0	0
%	0	1	1	2	0	0	0	0	0
Combined	1	1	2	1	3	0	0	2	3
%	1	1	2	2	4	0	0	2	3

*Statistically significant, $p<0.01$, Peto, et al., (1980)

promoter, 2-0-tetradecanoyl-phorbol-13-acetate (TPA), was applied topically to the shaved back of each mouse 3 times per week for 20 weeks. Animals were observed weekly for tumors, and sacrificed 52 weeks after the initial acrylamide administration. By all three routes of administration, a dose-response increase of squamous cell carcinomas and papillomas was observed in SENCAR mice treated with acrylamide and TPA (Table 8.5). The incidence of squamous cell papillomas or squamous cell carcinomas as a function of the route of exposure was: oral > i.p. > topical. There was a highly significant dose-response relationship for time to first tumor as well as the appearance of multiple tumors by all three routes of administration ($P<<0.01$). Acrylamide ppeared to be roughly as potent as ethyl carbamate ($CH_2-CH_2-O-C-NH_2$), a structural analogue of acrylamide in initiating skin tumors. However, acrylamide treatment alone (without TPA) did not result in an increased incidence of skin tumors in the SENCAR mice under the conditions of the test (Bull et al. 1984a).

In a combined skin papillona/lung adenoma assay, significant increases in skin and lung tumor incidences were noted in SENCAR mice administered 50 mg/kg of acrylamide by a single i.p. injection followed by triweekly application of 1.0 ug TPA for 20 weeks (Robinson et al. 1986).

The tumorigenic activity of acrylamide in the skin and lung has also been studied on Swiss-ICR mice (Bull et al. 1984b). Acrylamide dissolved in water was administered by gavage in doses

Table 8.5. TUMOR-BEARING ANIMALS AND CLASSIFICATION OF HISTOLOGICALLY EXAMINED SKIN TUMORS IN SENCAR MICE INITIATED WITH ACRYLAMIDE BY VARIOUS ROUTES[a]

Total Dose (mg/kg)	Route of Administration	TPA	Cumulative No. of Tumor Bearing Animals[6]/No. of Animals Initiated	No. of Squamous Cell Papillomas/ No. of Animals Examined	No. of Squamous Cell Carcinoma/No. of Animals Examined	% of Animals Bearing Squamous Cell Carcinomas
Ethyl Carbamate 300	p.o.	+	8/20	0/16[e]	3/16[e]	18.8
Acrylamide						
0[c]	p.o.	+	2/40	0/34	0/34	0
75	p.o.	+	12/40	3/35	2/35	5.7
150	p.o.	+	23/40	8/33	7/33	21.2
300	P.O.	+	30/40	11/38	6/38	15.8
300	p.o.	-	0/20	0/17	0/17	0
0[c]	i.p.	+	0/40	0/35	0/35	0
75	i.p.	+	10/40	2/38	2/38	5.2
150	i.p.	+	13/40	3/36	4/36	11.1
300	i.p.	+	21/40	6/35	4/35	11.4
300	i.p.	-	0/20	0/17	0/17	0

Table 8.5. (Cont.) TUMOR-BEARING ANIMALS AND CLASSIFICATION OF HISTOLOGICALLY EXAMINED SKIN TUMORS IN SENCAR MICE INITIATED WITH ACRYLAMIDE BY VARIOUS ROUTES[a]

Total Dose (mg/kg)	Route of Administration	TPA	Cumulative No. of Tumor Bearing Animals[b]/No. of Animals Initiated	No. of Squamous Cell Papillomas/ No. of Animals Examined[e]	No. of Squamous Cell Carcinoma/No. of Animals Examined	% of Animals Bearing Squamous Cell Carcinomas
Acrylamide						
0[d]	topical	+	7/40	5/36	0/36	0
75	topical	+	4/40	3/38	1/38	2.6
150	topical	+	11/40	3/35	2/35	5.7
300	topical	+	18/40	2/34	3/34	8.8
300	topical	-	0/20	0/20	0/20	0

[a]Source: Bull et al.; 1984a

[b]to be included in the cumulative count, the animal must have had a lesion. 1 mm in diameter, for 3 consecutive weekly observations.

[c]Distilled deionized water was administered in the same volume (0.2ml/mouse) and frequency as used in experimental groups

[d]Ethanol was administerd topically in the same volume (0.2ml/mouse) and frequency as used for experimental groups.

[e]Number of animals available for histological examination following death, or at termination of the experiment at 1 year; number excludes heavily autolyzed and cannabalized animals.

of 12.5, 25, or 50 mg/kg 6 times over a 2-week period. The total dose administered was 75, 150, or 300 mg/kg to 40 female mice per dose level. Two weeks after dosing with acrylamide, 2.5 ug of TPA was applied 3 times a week for a period of 20 weeks to the shaved back of the mice. The mice were observed weekly for the development of skin tumors and after one year for the development of lung tumors. Acrylamide was found to initiate squamous cell adenomas and carcinomas in the skin and to increase the yield of adenomas and carcinomas in the lung. (See Tables 8.6 and 8.7.) Skin tumor development was dependent on TPA promotion whereas lung tumor induction was not. As shown in Table 8.6, treatment with acrylamide followed by TPA promotion resulted in a dose-related increase in squamous cell carcinoma; at the high-dose level this increase in carcinomas, and also squamous cell papillomas, was statistically significant ($P<0.05$). Only one carcinoma was observed in the acrylamide-treated group that did not receive TPA. Note that ethyl carbamate followed by TPA promotion was somewhat less potent than acrylamide plus TPA in increasing the number of skin tumor-bearing animals as well as the number of skin tumors per animal. The yield of alveolar bronchiolar adenomas and carcinomas (combined) was increased in a dose-related manner ($P<0.03$) by acrylamide treatment, as shown in Table 8.7., and this increase was observed in the absence or the present of TPA promotion. As also shown in Table 8.7, ethyl carbamate treatment yielded a slightly higher combined yield of alveolar bronchiolar adenomas and carcinomas than did acrylamide treatment.

Table 8.6. TUMOR INITIATING ACTIVITY OF ACRYLAMIDE IN THE SKIN OF FEMALE SWISS-ICR MICE[a]

Chemical	Dose[b] (mg/kg)	TPA[c]	Cumulative No. of Tumor Bearing Animals[d]	Cumulative No. Tumors/Animals	Histological Classification[e] of Squamous Cell Tumors at Autopsy	
					Papilloma	Carcinoma
Distilled H_2O	0.2 ml	+	0/40 (35)	0	0	0
Acrylamide	75	+	4/40 (36)	0.10	1	1
	150	+	4/40 (32)	0.13	0	3
	300	+	13/40 (32)	0.43	6	4
	300	-	10/40[E] (36)	0.03	0	1
Ethyl Carbamate	300	+	4/40 (33)	0.18	2	3

[a]Source: Bull et al., 1984b

[b]Dose was administered orally in six divided portions over a 2-week period to the total indicated.

cTPA was applied at a dose 2.5 ug/mouse in acetone 3 times/week for 20 weeks beginning 2 weeks after the last dose of initiator.

[d]To be included in the cumulative count a 1 mm mass had to be present in the same location for 3 consecutive weekly observations. Numbers of animals begun in each group is indicated as the denominator of the ratio. Animals surving to terminaton of the experiment at 1 year included in parentheses.

[e]The difference between total tumors observed at autopsy versus the cumulative yield of tumors was related to the regression of papillomas.

[f]This value is probably 1/40 since it was stated in the text of the publication that only 1 tumor was found in this group.

Table 8.7. LUNG TUMOR INCIDENCE IN SWISS ICR-MICE TREATED WITH ACRYLAMIDE[a]

Chemical	Dose[b] (mg/kg)	TPA[c]	N[d]	Alveolar Bronchiolar Adenoma	Alveolar Bronchiolar Carcinoma	Total
Distilled H_2O	0.2 ml	+	36	3	1	4
Acrylamide	75	+	34	6	2	8
	150	+	36	5	1	6
	300	+	34	10	1	11
	300	-	36	4	10	14
Ethyl Carbamate	300	+	36	9	8	17

[a]Source: Bull et al., 1984b

[b]Dose was administered orally in 6 divided portions over a 2-week period to the total dose indicated.

[c]TPA was applied at a dose of 2.5 ug/mouse in acetone 3 times/week for 20 weeks beginning 2 weeks after the last dose of initiator.

[d]N = Number of animals available for autopsy without unacceptable levels of autolysis. Each group initially contained 40 animals.

Groups of 40 strain A/J mice (8 weeks old) per sex per dose level were administered acrylamide po at 6.25, 12.5, or 25.0 mg of acrylamide per kg of body weight 3 times per week for 8 weeks (Bull et al., 1984a). Ethyl carbamate was administered by the same regimen at doses of 10.5, 21.0, and 42 mg/kg. Mice were sacrificed at 9 months of age. Both acrylamide and ethyl carbamate increased the yield of lung adenomas in each sex in a dose-related manner; a dose-response relationship ($P<0.01$) was observed for both animals with tumors and the multiplicity of tumors for each chemical. The potency of ethyl carbamate was seven times higher than acrylamide per unit of dose in increasing the number of tumors per animal. These data were presented in a figure in Bull et al. (1984a), but are not presented in this review since the data were not tabulated.

Groups of 16 strain A/J mice (8 weeks old) per sex per dose were administered acrylamide by ip at 1, 3, 10, 30, or 60 mg/kg of acrylamide, 3 times per week for 8 weeks. Positive control groups received a singe ip injection of ethyl carbamate at 500 or 1000 mg/kg. Animals were sacrificed at 8 months of age. A dose-response relationship was observed for the number of lung adenomas ($P<<0.01$) produced by acrylamide treatment, as shown in Table 8.8. A higher dose of 60 mg acrylamide/kg was also attempted, but discontinued after the 11th injection due to frank peripheral neuropathy and decreased survival. Based on some additional testing, Bull et al. (1984a) concluded that acrylamide is slightly more potent in lung tumor production by the ip route

Table 8.8. EFFECTS OF ACRYLAMIDE ADMINISTERED INTRAPERITONEALLY ON DEVELOPMENT OF LUNG ADENOMAS IN A/J MICE[a]

Treatment	Sex	No. of Survivors/ Initial No. of Mice	Percent of Mice with Lung Tumors	Average No. of Lung Tumors/Mouse
None	M	16/16	31	0.31+0.48[b]
	F	14/16	50	0.50+0.52
	M, F	30/32	40	0.40+0.50
Distilled H_2O	M	16/16	13	0.06±0.25
	F	15/16	8	0.13±0.35
	M, F	31/32	10	0.10±0.30
Acrylamide				
1 mg/kg/day[c]	M	16/16	50	0.75±0.93
	F	17/17	35	0.35±0.93
	M, F	33/33	42	0.55±0.78
3 mg/kg/day[c]	M	16/16	38	0.69±1.03
	F	17/17	53	0.88±1.11
	M, F	33/33	46	0.79±1.05
10 mg/kg/day[c]	M	17/17	59	0.88±0.99
	F	14/15	79	1.57±1.79
	M, F	31/32	68	1.19±1.40
30 mg/kd/day[c]	M	15/16	93	1.87±1.55
	F	15/16	93	2.53±1.46
	M, F	30/32	93	2.20±1.52

[a]Source: Bull et al., 1984a

[b]Mean ± S.D.

[c]Given 3 days/week for 8 weeks

than by the po route. since A/J mice usually develop from 1 to 2 lung tumors per animal at 1.5 years of age, acrylamide treatment accelerated tumorigenesis since this yield was attained in treated animals at a younger age.

8.6. Other Data to Support the Carcinogenicity Conclusion

8.6.1. Mutagenicity Data

Acrylamide has been shown to be a clastogenic agent in both *in vivo* and *in vitro* studies, and this effect is more pronounced in germ cells than in somatic cells (for details see mutagenicity section). Evidence presently available has not shown acrylamide to be an effective point (gene) mutagen. Additional evidence suggesting that acrylamide can interact with DNA in such a way as to produce mutation and possible cancer include DNA damage and repair effects of acrylamide and a study that shows that acrylamide binds to DNA. A positive *in vitro* cell transformation study (and possibly the amplification of SV40 DNA inserts in SV-40-transformed Chinese hamster cells treated with acrylamide) further supports the carcinogenic potential of acrylamide.

8.6.2. Absorption, Distribution and Metabolism Data

Absorption of acrylamide has been shown to occur via the oral and dermal routes of administration. After absorption, acrylamide rapidly equilibrates in the body, and does not accumulate in tissues other than the red blood cells (presumably

acrylamide binds to the sulfhydryl groups of hemoglobin). Acrylamide is rapidly metabolized in the body, primarily by conjugation with glutathione, and excreted. The mechanism of carcinogenic action is most likely via direct alkylation by Michael addition, or via metabolism to the epoxide, but the data do not conclusively support one of these two possible mechanisms for the observed carcinogenic or mutagenic effects of acrylamide. (For details see metabolism section.)

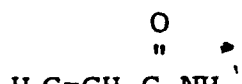
As mentioned above in the discussion of Bull et al. (1984a) acrylamide (followed by topically applied TPA) was shown to be a more potent skin tumor initiator when administered orally than when applied topically to SENCAR mice. (See also Table 8.5.) Carlson and Weaver (1985) compared the ^{14}C-acrylamide distribution, and binding to macromolecules, in SENCAR mice following oral and topical administration. Comparing the two routes, comparable concentrations were observed in all tissues except the skin where both the total amount of ^{14}C-acrylamide, and the amount bound to DNA, was much greater after topical administration than that observed after oral administration. Therefore, the effect of route on skin tumor formation cannot be explained on the basis of either a difference in distribution to, or binding to DNA in, the target organ.

8.6.3. Structure-Activity Relationship (SAR) Data

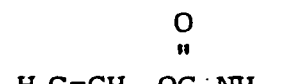
The structure-activity relationship of acrylamide and vinyl carbamate (the presumed proximate carcinogen form of ethyl

carbamate) has been suggested by Bull et al., (1984a) who showed that ethyl carbamate and acrylamide behaved similarly in tumorigenicity studies. (See 8.2.3 for discussion.) The authors argued that ethyl carbamate acted through vinyl carbamate as an intermediate, and suggested the structural similarity of vinyl carbamate an acrylamide (note that both are alpha, beta-unsaturated nitrogen-containing molecules).

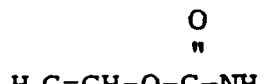
The sites of tumors observed in test animals administered ethyl carbamate, acrylamide and a third chemical, acrylonitrile show some similarities, and some dissimilarities (IARC, 1979). The amount of tumor site concordance observed suggests some similarity in the carcinogenic action of these three chemicals.



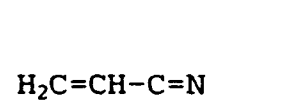
acrylamide



ethyl carbamate



vinyl carbamate



acrylonitrile

8.7. Data for Risk Assessment

Table 8.1 summarizes the tumor incidence data to be considered for preliminary risk assessment purposes. Of all the sites considered, testes, thyroid, and mammary gland were identified as those to be used for preliminary risk assessment of

individual sites should one be deemed necessary. Testes was chosen because the tumors are all malignant and are significantly increased at a dose level below the highest dose. Thyroid gland was chosen because the tumors were significantly increased for both sexes indicating the sensitivity of this site to chemical action across sexes. Mammary gland was chosen because benign, and combined benign and malignant tumors, were statistically significantly elevated at both the high dose, and the next lowest dose.

The EPA guidelines for cancer risk assessment recommend pooling tumor incidence data for purposes of risk assessment, since risk numbers derived from site-specific tumor incidence data may not be predictive of (and may in fact underestimate) "whole-body" risks that are determined using the pooled individual animal data. The dose-response curves for each sex based on the pooled tumor incidence (benign and malignant) data is the data set of choice for risk assessment. Further, a second pool was created for each sex to allow consideration of the contribution of malignancies alone to the overall risk estimates. Table 8.2. summarizes the pooled tumor incidence data.

In order for tumors at a particular site to be added into the pool, the tumor site must have been statistically significant at least at the high-dose level (treated vs. control). The first pool contains males having tumors of the testes, thyroid, or adrenal gland. For the adrenal gland, only the (benign) adenomas were considered since neither malignant alone, nor adenoma and

adenocarcinoma combined, were statistically significant. The second male pool contains testes mesothelioma only. The third pool contains females having tumors of the thyroid (combined), mammary gland (combined), CNS (tumors only, no "proliferations"), uterus (adenocarcinoma) and oral (combined). The fourth pool contains females having malignant tumors of the thyroid, mammary gland, CNS, and uterus. Tumors of the clitoral gland in females are not included in the pooled tumor incidence data because only a very low number of tissues were examined in each dose group.

9. EXPOSURE

9.1. Manufacture

9.1.1. Production Sites, Quantities, and Trends

Acrylamide is produced in the United States by three chemical manufacturers (Table 9-1; Figure 9-1). Total domestic production capacity as of January 1, 1985 was 225 X 10^6 pounds (lbs.). In 1985, 140 X 10^6 lb. of acrylamide were produced in the United States, approximately 62% of production capacity. The production volume increased at an average annual rate of 15.3% between 1978 and 1984; however, future increase in demand is estimated to average 4% a year through 1989. (Table 9-2).

American Cyanamid Co. has the largest capacity for acrylamide production (110 X 10^6 lbs.), accounting for 49% of total domestic capacity (Table 9-1).

Less than 2 X 10^6 pounds of acrylamide are imported, mostly for grouting purposes.

9.1.2. Production Methods and Processes

The major commercial production methods that have been used to manufacture acrylamide are:

1. Sulfate process and
2. Catalytic hydration process

Industrial production of acrylamide was initiated by American Cyanamid Co. in 1954, using the sulfate process (Matsuda, 1977). The sulfate process was the major production

Table 9-1. Acrylamide Manufacturers, Locations, and Annual Production Capacities

Manufacturer	Location	Annual Capacity (X 10^6 lbs.)	Percent of Total
American Cyanamid Company	Linden, NJ*	80	36
	Avonale, LA	30	13
Dow Chemical Company	Midland, MI	100	44
Nalco Chemical Company	Garyville, LA	15	7
TOTAL		225	100

U.S. EPA, 1986.

*This facility was closed in 1986 (American Cyanamid Company, 1986).

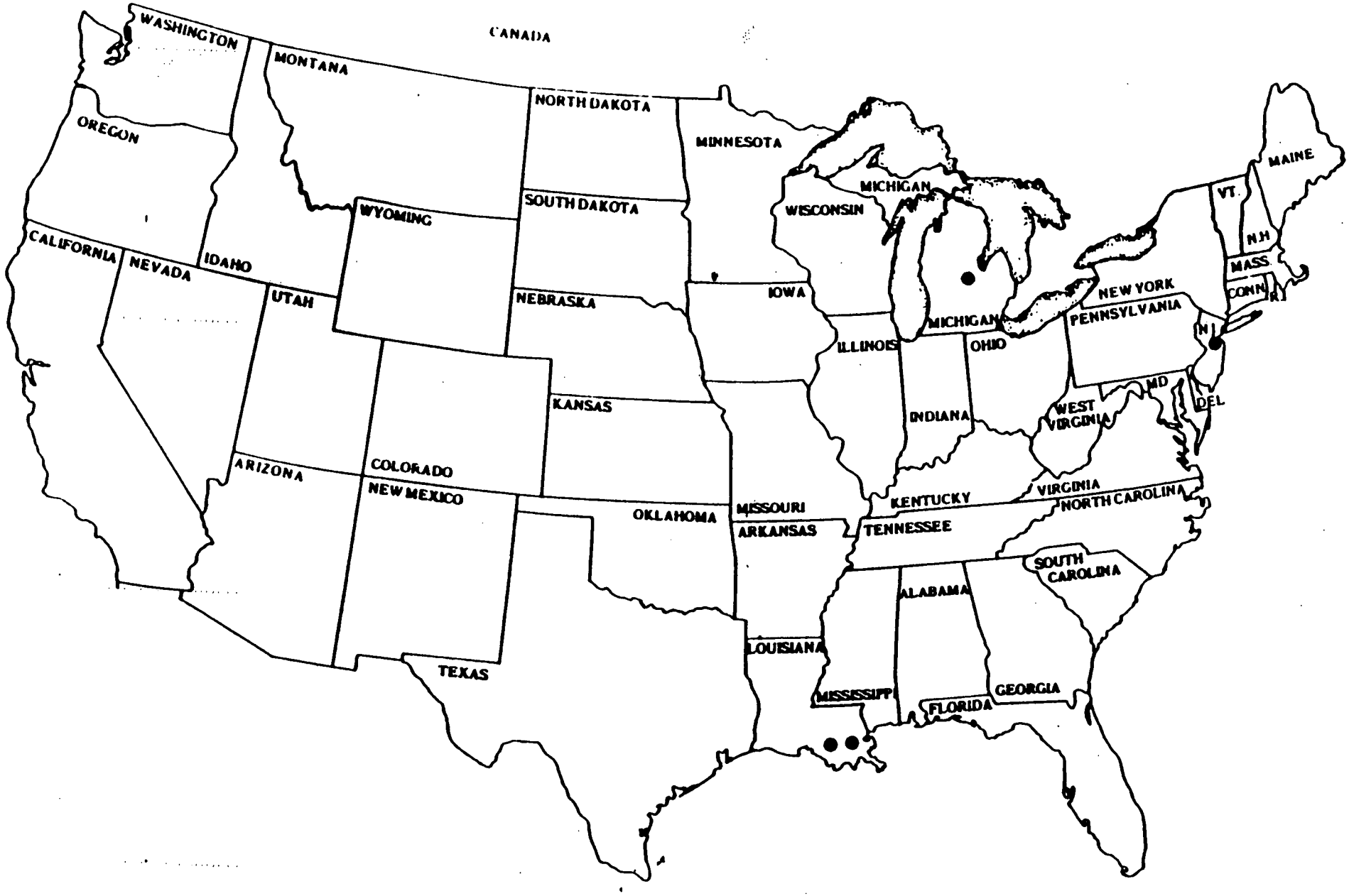


Figure 9-1. Production sites for acrylamide. From Environ. Sci. Eng. (1981).

Table 9-2. Annual Acrylamide Production Rates

Year	Acrylamide Production ($X 10^6$ lbs.)
1979	75
1980	77
1981	82
1982	86
1983	95
1984	130
1985	140
1989	164 (estimate)

U.S. EPA, 1986.

method until the early 1970s (Davis et al., 1976). In this process, approximately equimolar quantities of acrylonitrile, sulfuric acid, and water are reacted to produce acrylamide sulfate (Figure 9-2). The acrylamide sulfate is then neutralized by reaction with bases (ammonia, calcium hydroxide, or sodium carbonate) and separation by filtration. This procedure is expressed by the following reaction formulae:

$$CH_2{=}CHCN + H_2O + H_2SO4 \longrightarrow CH_2{=}CHCONH_2 \cdot H_2SO_4$$

Acrylonitrile Sulfuric Acid Acrylamide Sulfate

$$CH_2{=}CHCONH_2 \quad H_2SO_4 + 2NH_3 \longrightarrow CH_2{=}CHCONH_2 + (NH_4)_2SO_4$$

Acrylamide Sulfate Ammonia Acrylamide Ammonium Sulfate

Finally, the acrylamide is recovered by crystallization from the filtrate. Dow Chemical Co. (1980), however, separated acrylamide from the sulfate salt by passing the acrylamide sulfate through a bed of cation-exchange resin (Figure 9-2).

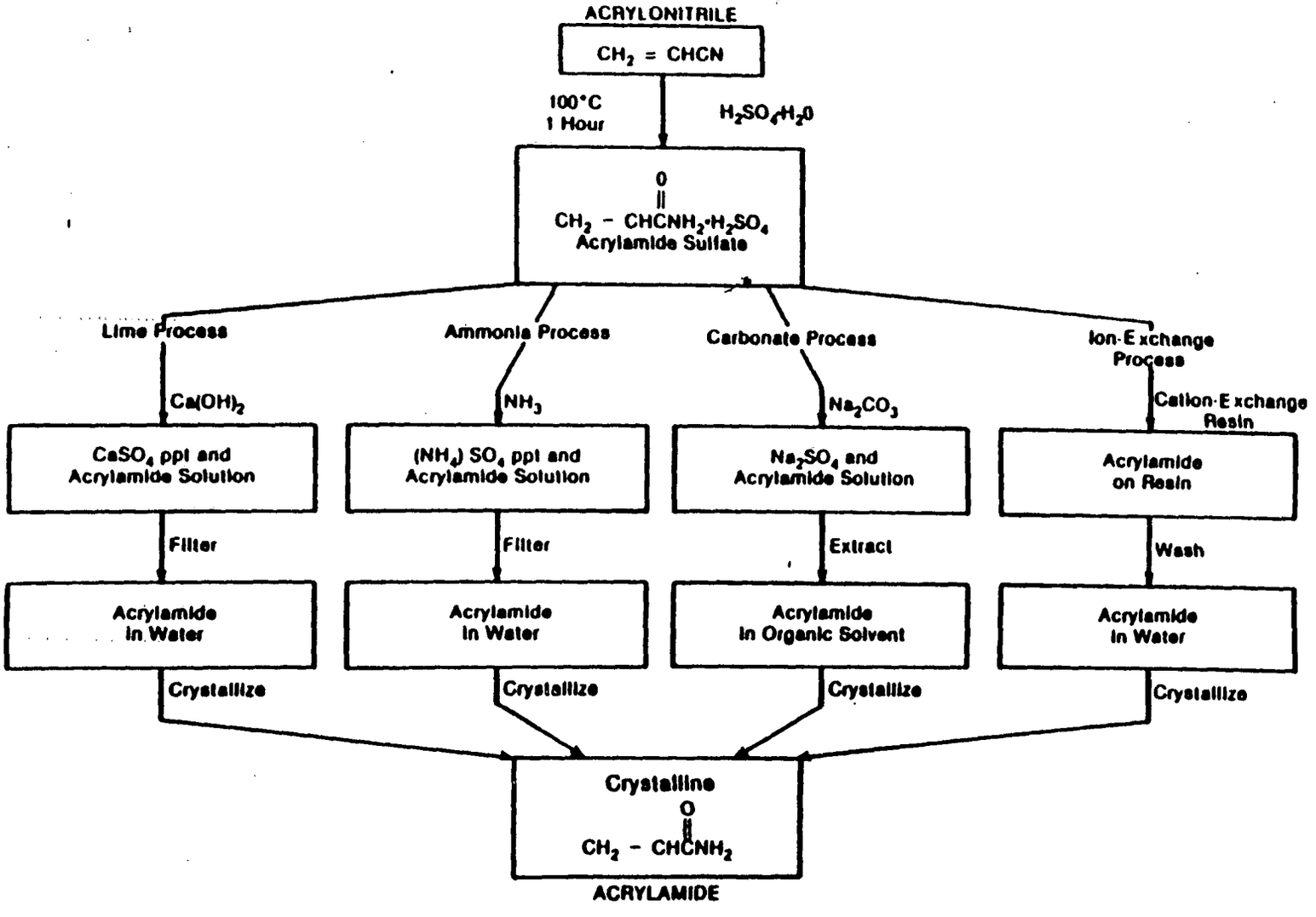


Figure 9-2. Sulfate process for acrylamide. From Environ. Sci. Eng. (1981).

During the early 1970s, the sulfate process for acrylamide manufacture was replaced by the catalytic hydration process (Davis et al., 1976; Matsuda, 1977). In this process (Figure 9-3), an aqueous solution of acrylonitrile is passed through a bed of copper-based catalyst. This method is expressed by the following reaction formula:

$$CH_2{=}CHCN + H_2O \longrightarrow CH_2{=}CHCONH_2$$

Acrylonitrile — Cu Catalyst — Acrylamide

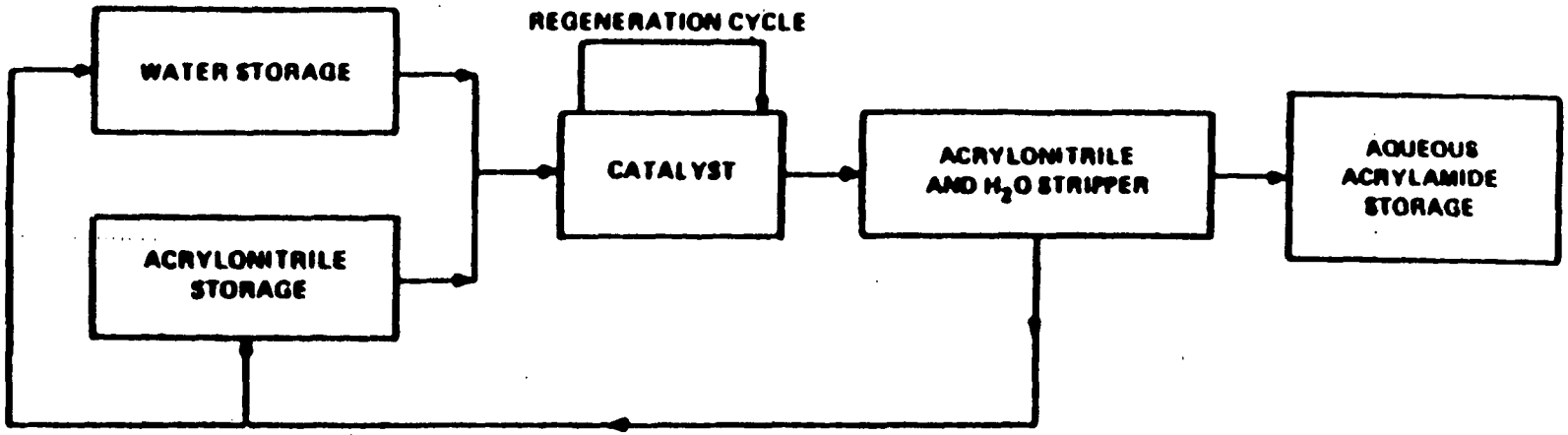


Figure 9-3. Catalytic hydration process for acrylamide. From Environ. Sci. Eng. (1981).

The catalyst is washed and regenerated in a regeneration cycle. The acrylamide solution is concentrated and stripped of unreacted acrylonitrile by distillation. The distillate from this process is recycled to the reactor. Water is added to the acrylamide solution to reach the desired concentration, usually 30 to 50 percent acrylamide by weight (Davis et al., 1976).

9.2. Uses

9.2.1. Acrylamide Monomer

Acrylamide consumption in 1984 was estimated to be about 130 million pounds. Approximately 95% of all acrylamide produced was used to manufacture polymers of acrylamide. These polymers, frequently called polyacrylamides, are widely used in water treatment applications; in the petroleum industry, primarily in enhanced oil recovery; in pulp and paper production; and in mineral processing. The remaining 5% of acrylamide production was consumed in several small applications. Table 9-3 lists the 1984 end use pattern for acrylamide.

Table 9-3. Acrylamide End Use Pattern, 1984

End Use	Form	Percent of Total Market
Water Treatment	Polymer	45
Petroleum	Polymer	20
Pulp and Paper	Polymer	20
Mineral Processing	Polymer	10
Miscellaneous	Polymer/Monomer	5

Source: CMR, 1985

Overall, acrylamide demand is forecasted to grow 4% per year through 1989 (CMR, 1985).

9.2.2. Polyacrylamide Polymers

Acrylamide undergoes polymerization to produce a group of versatile, synthetic polymers called polyacrylamides. Polymers of commercial significance include those derived from polymerization of acrylamide with itself (homopolymer) and those derived from polymerization with other comonomers (copolymers) (Schwayer, 1981).

While a large number of commercial polyacrylamides exist, they are usually classified according to their molecular structure, molecular weight (size), and the electrical charge they exhibit in water. Polyacrylamides are either low ($\leq$ 100,000 g/mole) or high ($\geq$ 1,000,000 g/mole) in molecular weight. The polymers are also categorized as either anionic, cationic, or nonionic, depending on whether they exhibit a negative, positive or no electric charge. The polymer's electric charge and molecular structure are determined by the particular method of preparation.

Polyacrylamides are readily soluble in water over a broad range of conditions. They can be engineered to fit a large number of uses (Davidson, 1980). Polyacrylamides have several properties which have led to their use in a wide variety of industries. Table 9-4 lists the key functions of polyacrylamides, sample commercial applications, and the industries currently using them. The specific polymer used in each application is determined by the performance requirements of the specific use and the characteristics of the polymer itself.

Table 9-4. Functions of Polyacrylamides

Function	Application	Industry
Flocculation	Solids recovery	Mining
	Waste removal	Sewage
	Water clarification	General
	Retention aid	Paper
	Drainage aid	Paper
Rheology Control	Waterflooding	Petroleum
	Viscous drag reduction	Petroleum
		Fire fighting
		Irrigation pumping
Adhesion	Dry strength	Paper
	Wallboard cementing	Construction

Source: Davidson, 1980

The general properties of polyacrylamides are derived from the chemical nature and structure of the acrylamide monomer. Commercially available polyacrylamides are subdivided into two major classes based upon their electrical charge. Anionic or negatively charged polyacrylamides are obtained from the hydrolysis of the acrylamide's amide group or through copolymerization of acrylamide with acrylic acid. Cationic or positively charged polyacrylamides are produced via copolymerization of acrylamide with other chemicals, such as formaldehyde and amines.

9.2.2.1. Polyacrylamide Manufacturing Process

Anionic polyacrylamides are produced by one of two processes. The first involves simultaneous polymerization and

hydrolysis of acrylamide. Sodium hydroxide or sodium carbonate is added to an aqueous solution of acrylamide to give the desired degree of hydrolysis. The acrylamide is then polymerized. The resulting polymer contains carboxyl groups which ionize in water to give the polymer its anionic character. Alternatively, anionic polyacrylamides are produced by co-polymerization of acrylamide with acrylic acid or acrylate salts. Polymers of comparable molecular weights appear to function similarly regardless of the method of preparation (Schwayer, 1981).

Cationic polyacrylamides are prepared by one of two methods. In the first, acrylamide is first polymerized to produce a homopolymer which is then reacted with formaldehyde and dimethylamide to form a cationic aminomethylated polyacrylamide. Alternatively, acrylamide can be co-polymerized with a variety of monomers which impart cationic functionality. Some of the more important products are diallyldimethyl ammonium chloride-acrylamide copolymer, aminomethacrylate-acrylamide copolymer, and dimethyl-aminoethyl methacrylate-acrylamide copolymer (Kirk-Othmer, 1982).

Polymerization of acrylamide occurs under a number of conditions. The most commonly used process is aqueous solution polymerization. Figure 9-4 depicts the important steps in the manufacture of liquid polyacrylamide by this method. The polymerization reaction can be initiated by peroxides, persulfates, azo compounds, or redox reagents. The molecular weight of the final polymer can be varied by several techniques,

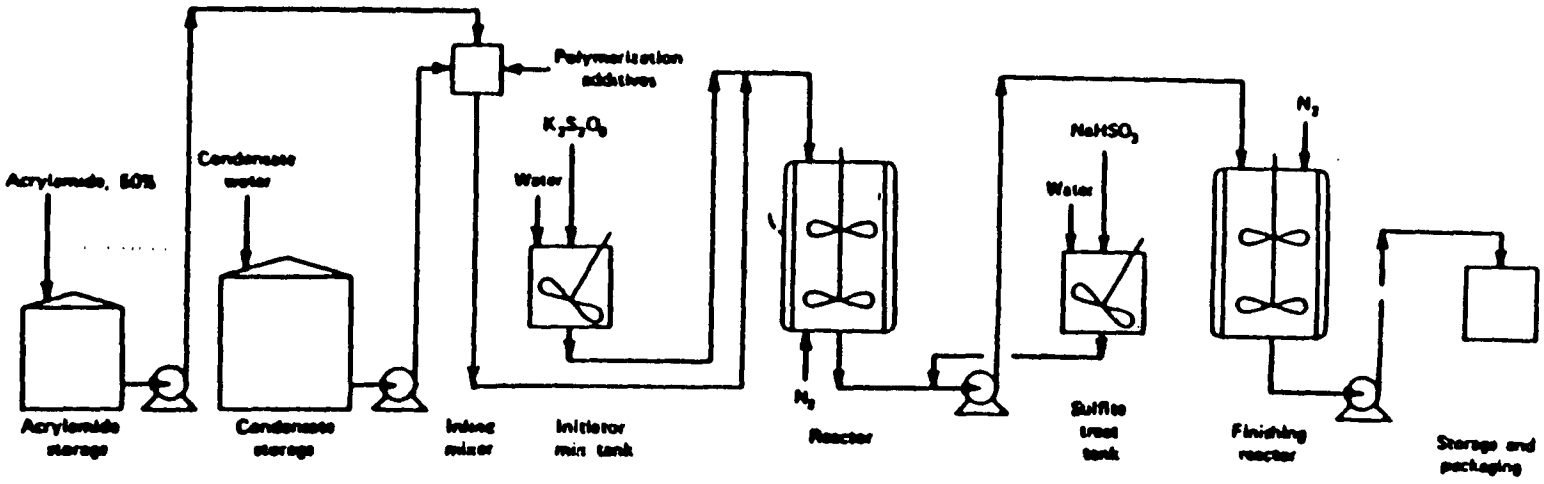


Figure 9-4. General diagram of solution polymerization, liquid products process (Kirk-Othmer, 1978).

including acrylamide concentration, initiator concentration, polymerization temperature, polymer chain-transfer agents, and electrolyte concentration (Kirk-Othmer, 1978). The main drying techniques used to manufacture solid high-molecular weight polyacrylamides from aqueous solutions are thermal sheet drying and solvent precipitation (Kirk-Othmer, 1978).

9.2.3 Polyacrylamide End Uses

9.2.3.1 Water Treatment Applications

Water treatment applications represent the largest market for polyacrylamide. It is estimated that these uses currently consume about 45% of the acrylamide produced annually in the United States (CMR, 1985). Applications include the use of polyacrylamides in municipal water and wastewater treatment plants, in sludge conditioning operations, and in industrial raw and wastewater treatment plants. In addition to being the largest use, these applications probably represent the most widespread use of polyacrylamides. In general, the use of polyacrylamides in these uses is based upon the polymers' ability to facilitate the removal of solids from water.

9.2.3.2. Pulp and Paper Applications

Polyacrylamide use in pulp and paper applications accounted for approximately 20% of acrylamide consumption in 1984 (CMR, 1985). These uses are based on polyacrylamide's excellent flocculation and chemical bonding properties (Bikales, 1973;

Kirk-Othmer, 1978). Water-soluble polyacrylamides are used for the following purposes.

- To improve the dry-strength of paper sheet;
- To increase the retention of pigments, inorganic fillers, and other small particulate matter that are added to paper sheet; and
- To improve the drainage of water during paper formation.

(American Cyanamid, 1969; Bikales, 1967; Kirk-Othmer, 1978).

9.2.3.3. Petroleum Applications

The petroleum industry uses polyacrylamides for several applications. The largest use is in enhanced oil recovery processes. They are also used in drilling-mud additives and in lubricant additives (Chem Purch, 1983; Kirk-Othmer, 1982a and b; Nyquist and Yocum, 1973).

9.2.3.4. Mining and Mineral Processing Applications

Polyacrylamide applications in mining and mineral processing account for 10% of the consumption of acrylamide. High-molecular weight polyacrylamides are used as flocculants for the recovery of valuable metals from ores, the recovery of tailings and slimes, and the clarification of wastewater. Low-molecular weight polyacrylamides function as sequestering and antiprecipitating agents, which reduce scale deposits in

equipment and keep solids suspended in solution for subsequent removal (American Cyanamid, 1969; Bikales, 1968; Nyquist and Yocum, 1973).

9.2.4. Other Uses of Acrylamide

9.2.4.1. Acrylamide Grouts

Acrylamide has been used in the formulation of chemical grouting agents. Chemical grouting is the practice of injecting chemicals into soil, rock or concrete formations to aggregate them to form a water barrier. Grouting is normally employed when it is desirable to restrict or redirect the flow of water through a formation or to improve formation strength. Grouting has been performed during the construction or rehabilitation of dams, buildings, tunnels, mine shafts, and other structures (Kirk-Othmer, 1979).

The predominant use of acrylamide grouts is in stopping water infiltration into sewers. It is estimated that about one-half million pounds of acrylamide grout is used annually for this purpose (Geochem, 1985). Specially-designed injection equipment is moved through underground sewer lines, and positioned near leaking sewer joints through the use of video cameras. After treatment, the cameras provide an immediate determination as to the effectiveness of the seal (Kirk-Othmer, 1979) (some grout is injected through hand held injector guns, especially in manhole sealing operations). Smaller quantities of acrylamide grout are

believed to be used in repairing cracks in tunnels, mine shafts, and ground application (Celtite, 1985).

American Cyanamid had been the major domestic producer of acrylamide grout until the company terminated production in 1978. Imported grouts based on acrylamide are available from: Avanti International, Inc. (Webster, TX), Cues, Inc. (Orlando, FL), and Polymer Chemicals (Atlanta, GA). Avanti and Cues import and market an acrylamide grout produced in Japan by Nitto Chemical Company. Polymer Chemicals imports from a French company. Mitsubishi and Sumitomo Trading Companies act as agents for Nitto in these sales. Avanti markets the grout under the tradename AV-100 and Cues markets it under the trade name Q-Seal (Cues, 1985; Geochem, 1985). Both products are sold as dry powders (Karol, 1983).

Acrylamide-based grouts generally consist of a mixture of two monomers: acrylamide, which represents 95% of the mixture, and a cross-linking agent, such as methylene-bis-acrylamide, which represents the remaining 5%. In commercial use, an aqueous solution of the powdered acrylamide grout is prepared at the field location. The solids content of the solution can vary, but the solution is prepared so as to contain 10% solids when injected into the sewer line crack. An activator chemical, typically triethanolamine, is then mixed into the solution. A second solution is prepared consisting of an initiator or catalyst, typically ammonium persulfate, in water. Once the injection equipment is positioned at the area to be repaired, the

solutions are mixed and injected into the crack. Upon injection, the acrylamide polymerizes and the cross-linking agent binds the polymer chains together, converting the mixture into the gel, thus closing the hole in the sewer line (Geochem, 1985; Karol, 1983). The gels are considered permanent and are unaffected by exposure to chemicals, except for very strong acids and bases. The gels are, however, subject to mechanical deterioration when exposed to alternating drying and/or freezing cycles (Karol, 1983).

In gel form, the grout contains very little free acrylamide. When properly prepared, the gel produced from a 10% solution of grout contains less than 0.03% acrylamide. It is known that many microorganisms in the soil and natural water assimilate ungelled acrylamide (Karol, 1983).

9.2.4.2. Monomer Derivatives

The total number of applications of acrylamide and its derivatives are unknown. One important and growing use of acrylamide is in the textile industry.

Acrylamide is used to produce n-methylol acrylamide, which is used as a textile anti-creasing agent. In one patented process the n-methylol acrylamide is introduced into cellulosic fibers, such as cotton, and is cured with radiation. The irradiation step causes the double bond of the acrylamide derivative to cross-link with the cellulose. The use of this derivative in fabric treatment results in good resistance to

creasing and generally good abrasion resistance (Textile Res J, 1960).

A small amount of acrylamide is used in laboratories for the preparation of gels for electrophoresis separations.

9.3. Acrylamide Exposure

9.3.1. Populations Exposed

Plant workers at both acrylamide and polyacrylamide production facilities and those involved in applying acrylamide grout are exposed to the monomer. Exposure can result from bagging operations at solid acrylamide production plants (the one U.S. site producing solid acrylamide ceased the production in 1985), disposal of used containers, washing down or otherwise treating spilled monomer, reactor or transport vessel cleaning, or mixing dry grouting formulations in the field. Direct worker contact (skin and eyes) with either the liquid or solid chemical is the most likely (and perhaps more important) route of exposure, although the workplace air can be a source if the monomer has accumulated in any areas, particularly in concrete walls or floors. Workers are routinely advised to wear protective clothing, rubber gloves, goggles, and respirators, where necessary.

In addition to the persons described above, some laboratory personnel are exposed to acrylamide during the preparation of polyacrylamide gels for gel electrophoresis. Such exposure could mainly occur during weighing, mixing, and during the subsequent

use of the gel solution. The route of exposure could be either inhalation or dermal, although dermal exposure is more probable because crystalline acrylamide is rather hygroscopic.

There are no individual consumers of acrylamide or related products. Users consist of other companies purchasing monomer for use as a feedstock, municipalities or industries purchasing polyacrylamide products for any one of their multiple uses (see section 9.2.3). Thus, product exposure would be limited to industrial and municipal workers. The exposure would be through residual acrylamide in the polyacrylamide product. In the general population, persons in cities served by drinking water supplies treated with polyacrylamide flocculating agents and consumers of sugar may be exposed to acrylamide monomer, again from its occurrence as a residue in the polymer product.

Other portions of the general population that might be affected would be those people living in the vicinity of grouting applications for soil stabilization and dam repair and populations living near acrylamide production plants. Soil stabilization and dam repair are minor application areas compared to sewer rehabilitation and, therefore, population exposure would be expected to be minimal. Environmental monitoring at acrylamide production sites indicates little potential for exposure (GCA, 1980).

9.3.2. Population Estimates

Table 9-5 presents estimates of the number of persons exposed during the manufacturing, processing, and use of acrylamide or acrylamide-based products.

NIOSH estimates that between 700 and 1,000 workers are potentially exposed to acrylamide in the manufacture of acrylamide monomer and polymers, based on reporting from the acrylamide manufacturers (NIOSH, 1985). The NIOSH, National Occupational Hazard Survey lists the estimated total number of U.S. workers who are potentially exposed to acrylamide to be approximately 10,000 employed in 27 occupations (NIOSH, 1980). Workers performing acrylamide grouting are estimated to number 1,800 (Abt Associates, 1990). In addition, at least 100,000 to 200,000 persons are potentially exposed to acrylamide in laboratories from the preparation and use of polyacrylamide gels for electrophoresis (Versar, 1986).

Indirect exposure from the use of polyacrylamide flocculants to treat drinking water ranges from 4.5 million to 30 million persons (Delpire, 1985). The former number is the most likely estimate given flocculant usage rates, polymer sales and household water consumption.

The number of persons exposed to acrylamide from the use of other polyacrylamide products is unknown.

Table 9-5. Estimates of the Number of Persons Exposed to Acrylamide

Exposure Category	Estimated Number Exposed
Manufacture at total of Four Facilities	< 100
Manufacture/Processing of Acrylamide monomer	500-1,000 as per Public Comments and EPA Review
Soil Grouting	1,800
National Occupational Hazard Survey -- Occupational Exposure -- 27 Occupations	10,000
Laboratory Personnel-- Gel Electrophoresis	100,000-200,000
Drinking Water	4,500,000-30,000,000
Sugar Consumption	Up to 230,000,000

9.4. Exposure Estimates

The exposure information presented below was obtained from recent EPA sponsored field studies and industry supplied data. When the first section 8(e) notice reported findings of statistically significant numbers of tumors in female rats, it was determined that the existing exposure information would be inadequate for risk assessment. Consequently, after reviewing the populations potentially exposed, it was decided that two categories should be monitored, manufacture and processing of acrylamide monomer and soil grouting to repair sewers. The two categories were selected because they have the greatest potential for significant dermal and inhalation exposure as opposed to persons potentially exposed to acrylamide from its presence as a residue in polyacrylamide products. Such exposures would be very low in many instances even assuming worst case scenarios (e.g., potable water treatment).

The first study by EPA examined acrylamide manufacturing and processing sites and one soil grouting site. This study was conducted by NIOSH under an interagency agreement. Subsequent monitoring by EPA at additional soil grouting site was prompted by the high dermal exposure potential reported by NIOSH. Other exposure estimates presented are based on usage rates of polyacrylamide products (drinking water treatment and sugar refining). Other users of polyacrylamide products were not extensively studied because the focus of the risk assessment is on persons exposed to acrylamide monomer. Drinking water

treatment and sugar refining were examined in limited detail because of the very large number of persons potentially exposed.

9.4.1. Acrylamide Manufacture and Processing

NIOSH conducted industrial hygiene surveys at all four domestic acrylamide manufacturing plants represented by three companies (American Cyanamid, Dow and NALCO). These plants also manufacture polyacrylamides. Area and personal air monitoring was performed as well as observations of work practices.

Six job classifications cover the workers in the acrylamide monomer and polymer production areas. These are monomer operators, polymer operators, monomer material handlers, polymer material handlers, maintenance workers, and utility operators. The monomer and polymer operators control the manufacturing process. A portion of their time is spent in the control room and the remainder is spent in the process area. The material handlers load bags, drums, trucks, and rail cars with the final product. Other workers in the acrylamide areas are the maintenance workers and the utility operators who supply water, electric power, and steam to the plant (Hill and Greife, 1986).

NIOSH collected a single, full period eight-hour personal sample for all workers who were potentially exposed to acrylamide. Eight-hour time-weighted averages (TWA) were calculated in order to compare exposures between manufacturing plants. Wipe samples were collected as an indication of possible dermal exposure.

The sampling and analysis was accomplished by collecting acrylamide vapor and particulate on a 37 mm mixed cellulose ester filter-pore size 0.8 um, Millipore type AA, followed by a silica gel tube SKC no. 226-10, 100/50 at a flow rate of 1 liter per minute. The collected material was then desorbed from the collection media with water and analyzed by high performance liquid chromatography with an ultraviolet detector. The method was validated at air concentrations of 0.03 to 0.6 mg acrylamide per cubic meter of air based on a sample size of 480 liters of air (American Cyanamid Company, 1981) (Hill and Greife, 1986).

Wipe samples were collected as an indication of non-respiratory exposures. The surfaces that were wiped included the exterior of gloves and hard hats, inside the face piece of respirators, desk tops in the control rooms, on cafeteria table tops, on laboratory bench tops, and on reactor vessels.

The air monitoring data for the 4 manufacturing sites are summarized in Tables 9-6, 9-7, and 9-8. Two personal air samples were taken at the grouting site which were 0.002 and 0.007 mg/m^3 as 9 hour TWA's. The mean is 0.005 mg/m^3.

In addition to the NIOSH data, Sobel et al. (1986) reported (Dow Chemical) 8 hour TWA levels in the monomer production area ranging from 0.1 to 1.0 mg/m^3 for pre-1957, 0.1 to 0.6 mg/m^3 from 1957 to 1970, and <0.1 mg/m^3 for post-1970. In the polymer production area, most jobs had polymer dust level of <2 mg/m^3 except for packagers and dryer operators who had levels >2 mg/m^3. If a maximum 1% residual acrylamide is assumed (Sobel et al.,

1986), acrylamide exposure for the polymer workers described above would be <0.02 mg/m^3 and >0.02 mg/m^3, respectively. These data agree well with the NIOSH results. Koppers (1979) reported acrylamide levels in an amino resin production area ranging from <0.1 to <0.6 mg/m^3 for an amino resin operator and <0.09 to <0.05 mg/m^3 for the assistant amino resin operator, and <1 mg/m^3 for the filter press operator. The < notation denotes analytical limit of detection (monitoring was performed to duplicate an OSHA compliance officer monitoring technique). Koppers reported that other monitoring indicated exposure potential to be less than the OSHA standard of 0.3 mg/m^3. Finally, Collins (1984) reported acrylamide exposure levels ranging from 0.08 to 0.70 mg/m^3 in the monomer production area, 0.03 to 0.2 mg/m^3 in the polymer production area, and 0.15 mg/m^3 for maintenance workers.

Based on the NIOSH survey, American Cyanamid's Linden, New Jersey site had the greatest range of acrylamide air levels. This was due mainly to the production of dry acrylamide. The highest levels were found in enclosed areas of the production building, which workers would only occasionally enter with a full-facepiece canister-type respirator for acrylamide dust and vapors (this site is no longer producing acrylamide). Unlike this site, the other sites had all or a portion of their production process equipment open to the ambient air. The acrylamide production equipment at American Cyanamid's other site is entirely outdoors. The production equipment at Dow and NALCO are within a building, but natural ventilation is provided.

Table 9-6. NIOSH Monitoring
American Cyanamid-Linden, NJ Site

JOB TITLE	NO. SAMPLED	PERSONAL AIR SAMPLING DATA ACTUAL CONC. RANGE IN mg/m^3	MEAN	SD	RANGE	MONOMER PRODUCTION 8 HOUR-TWA MEAN	SD
OPERATORS	6	.087-.238	.185	.055	.079-.227	.170	.053
UTILITY OPERATORS	2	.309-.410	.360	.071	.306-.392	.349	.061
MATERIAL HANDLERS	3	.026-.298	.156	.136	.017-.260	.138	.122
MAINTENANCE	4	.001-.080	.048	.034	.001-.073	.035	.029
TOTAL FOR MONOMER AREA	15	.001-.410	.166	.118	.001-.392	.152	.115

JOB TITLE	NO. SAMPLED	PERSONAL AIR SAMPLING DATA ACTUAL CONC. RANGE IN mg/m^3	MEAN	SD	RANGE	POLYMER PRODUCTION 8 HOUR-TWA MEAN	SD
OPERATORS	9	.012-.223	.082	.075	.011-.181	.069	.061
MATERIAL HANDLERS	3	.022-.043	.031	.011	.018-.035	.026	.009
TOTAL FOR POLYMER AREA	12	.012-.223	.069	.068	.011-.181	.059	.056

Table 9-6 Cont. NIOSH Monitoring
American Cyanamid-Avondale, LA Site

JOB TITLE	NO. SAMPLED	ACTUAL CONC. RANGE IN mg/m^3	ACTUAL CONC. MEAN	ACTUAL CONC. SD	8 HOUR-TWA RANGE	8 HOUR-TWA MEAN	8 HOUR-TWA SD
MONOMER PRODUCTION							
OPERATORS	5	.034-.050	.041	.006	.032-.046	.039	.005
MATERIAL HANDLERS	1	.517	-	-	.051	-	-
MAINTENANCE	3	.016-.157	.064	.081	.015-.132	.054	.067
TOTAL FOR MONOMER	9	.016-.157	.102	.161	.015-.132	.045	.035
POLYMER PRODUCTION							
OPERATORS	8	.055-.105	.074	.016	.044-.098	.065	.019
MATERIAL HANDLERS	1	.018	-	-	.017	-	-
TOTAL FOR POLYMER	9	.018-.105	.068	.024	.017-.098	.060	.024

Table 9-7. NIOSH Monitoring
Dow Chemical Co.-Midland, MI Site

PERSONAL AIR MONITORING DATA
MONOMER PRODUCTION

JOB TITLE	NO. SAMPLED	ACTUAL CONC. mg/m³ RANGE	MEAN	SD	8 HOUR-TWA RANGE	MEAN	SD
Operators	8	.001-.119	.062	.033	.001-.113	.049	.032
Lab Tech	1	.053	.053	--	.049	.049	--
Total for Monomer	9	.001-.119	.061	.033	.001-.113	.0487	.030

PERSONAL AIR MONITORING DATA
POLYMER PRODUCTION

JOB TITLE	NO. SAMPLED	ACTUAL CONC. mg/m³ RANGE	MEAN	SD	8 HOUR-TWA RANGE	MEAN	SD
Operators	10	.001-.024	.008	.007	.001-.021	.007	.006
Maintenance	5	.001-.012	.005	.004	.001-.011	.005	.004
Lab Tech	1	.015	.015	--	.014	.014	--
Shipping Clerk	1	.004	.004	--	.004	.004	--
Total for Polymer	17	.001-.024	.007	.006	.001-.021	.007	.005

Table 9-8. NIOSH Monitoring
Nalco Chemical Co.-Garyville, LA Site

PERSONAL AIR MONITORING DATA
MONOMER AND POLYMER PRODUCTION

		ACTUAL CONC. mg/m^3			8 HOUR-TWA		
JOB TITLE	NO. SAMPLED	RANGE	MEAN	SD	RANGE	MEAN	SD
Operators	9	.003-.012	.006	.003	.003-.012	.006	.003
Utility Operators	2	.004	.004	0	.004	.004	0
Lab Tech	2	.003-.004	.004	.001	.003-.004	.004	.001
Material Handlers	4	.003-.005	.004	.001	.003-.005	.004	.001
Maintenance	2	.011-.014	.013	.002	.005-.006	.006	.001
Total	19	.003-.014	.006	.003	.003-.012	.005	.002

ND below .0035 mg/m^3

Monomer operators at American Cyanamid's sites and at Dow had a mean exposure level twice that of the polymer operators. Two utility operators at the Linden, New Jersey site had eight-hour TWA exposures above the OSHA standard. The company performed side-by-side sampling during the NIOSH survey, and confirmed the utility operators' exposure levels. The higher levels could not be explained, because their job duties do not involve direct exposure to acrylamide. Subsequent monitoring of these workers by the company revealed much lower exposure levels. Further monitoring of utility operators is necessary to determine sources of exposure. Monomer material handlers can have brief, high exposures when loading trucks or rail cars with acrylamide solution if they fail to wear personal protective equipment. The workers that were observed wore full-facepiece respirators, neoprene gloves, and an apron during this operation.

During the study, only minor maintenance occurred at each facility. However, these maintenance workers may potentially be exposed to airborne levels of acrylamide above the OSHA permissible exposure limit or they may be exposed by dermal contact. At the NALCO site, the major cleaning of the reactor equipment was performed by workers from a small contracting firm rather than in-house personnel.

The wipe samples were collected from personal protective equipment and workplace surfaces where skin contact was most likely. Nearly all of the wipe samples had no detectable acrylamide, except for a wipe sample collected on the exterior of

a polyacrylamide reactor vessel which had 30 ug/sample, a laboratory counter top which had 3.2 ug/sample, and a door handle which had 0.9 ug/sample (Hill and Greife, 1986).

9.4.2. Soil Grouting

Although soil grouting is a minor use of acrylamide, it presents the potential for high dermal exposure. As part of the NIOSH field investigation, a sewer grouting repair site was investigated. Subsequently, EPA carried out a field monitoring survey at four sewer grouting sites (MRI, 1987). At the sewer line repair site surveyed by NIOSH, two employees performed the repairs with the use of two service trucks. One service truck held the grouting equipment (hoses, pumps, mixing tanks, and video monitoring equipment), while the other truck contained hoses and a water supply. The workmen first assembled the packer, which was lowered into the manhole and then passed through the sewer. (See Figures 9-5 and 9-6.) It was positioned by the use of cables and a video camera viewed from the truck's control panel. At the site of a leak, both ends of the packer were inflated, isolating the leaking joint. In the service truck, an employee poured the grouting material, which was 95% acrylamide and 5% methylene-bis-acrylamide, into a mixing tank containing water. The acrylamide was bagged with an inner liner bag that could be placed below the water level of the mixing tank to prevent dust particles from escaping into the air. Acrylamide solution, along with a catalyst, was injected under pressure from

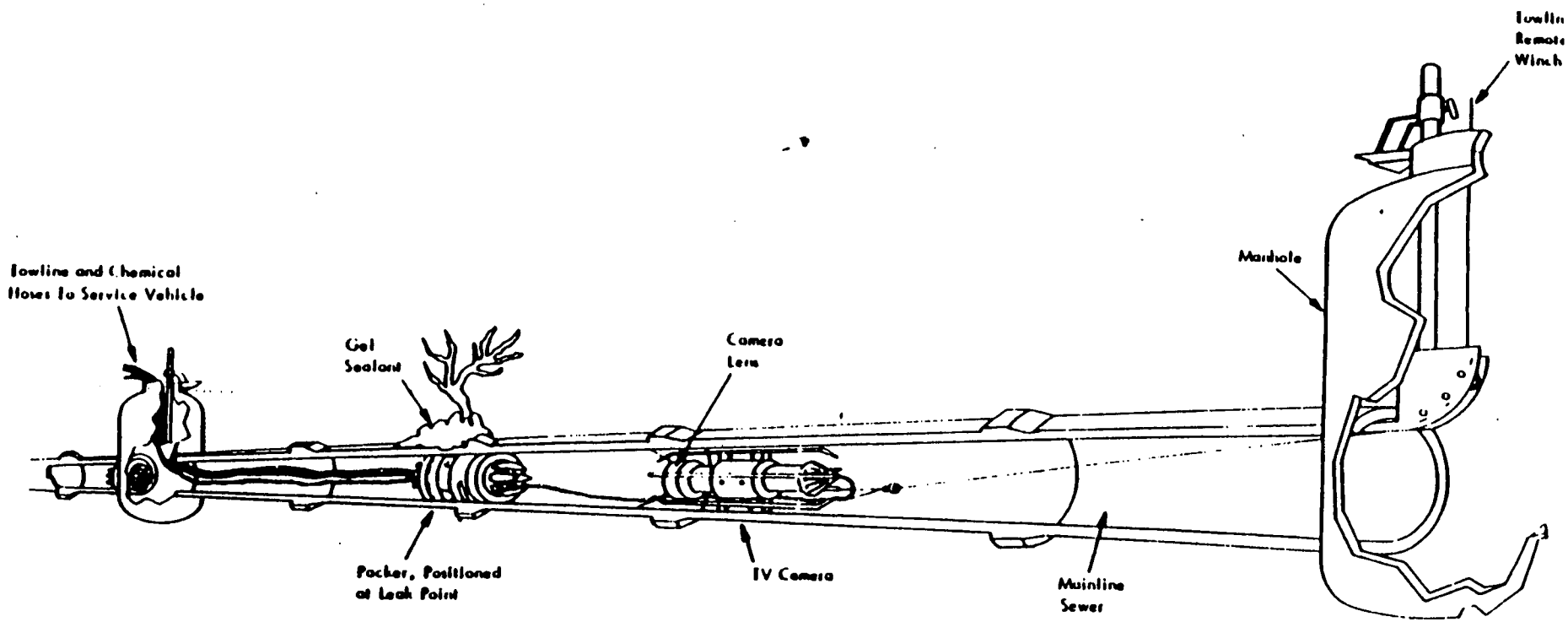


Figure 9-5. Sleeve packer and camera assembly inside mainline sewer.

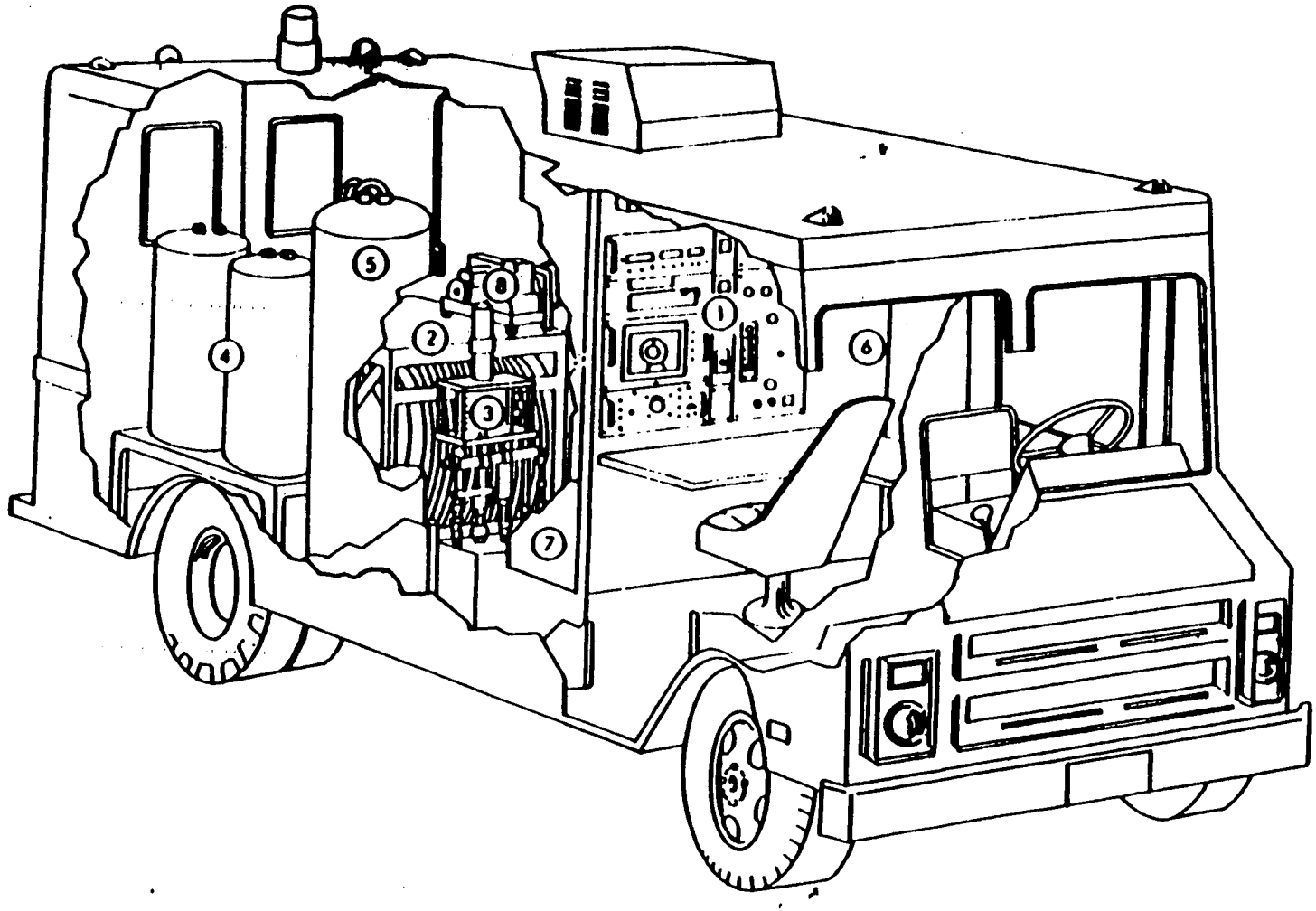


Figure 9-6, Typical line maintenance vehicle showing grouting equipment.

the service truck via hoses to the center of the packer and forced into the surrounding soil. The monomer polymerized to form a water-impermeable gel, sealing the leak. Once the leak was sealed, the packer was deflated and moved to the next joint. Two short-term exposure samples (18 minutes) were collected in a personal monitor on the individual who poured and mixed the dry acrylamide grout into the mixing tank. Nine-hour personal air samples levels were 0.002 and 0.007 mg/m^3. Two short term personal air samples, of 18 minute duration, collected during the pouring and mixing of the grout, did not show any detectable acrylamide. This may be due to the high moisture content of the acrylamide and the method of pouring the grout. Area air samples collected inside the service truck and the company garage revealed 0.009 and 0.001 mg/m^3, respectively.

Surface wipe samples collected from the control panel in the service van, on a safety cone placed on the road, and on the exterior of two rubber work gloves were free of acrylamide contamination. However, a wipe sample from the side of the grout mixing tank detected 44 ug/cm^2. The source of this contamination was apparent when a small amount of solid acrylamide was spilled on the tank and floor during the mixing. The spill was not cleaned up, and when the moisture in the grout evaporated, acrylamide could have become airborne as a dust or vapor. Other poor handling procedures observed, such as storing empty grouting bags and mixing cups on the floor, or only occasionally wearing

rubber gloves when handling the packer or washing the equipment, could lead to dermal contact with the acrylamide.

Another source of dermal acrylamide exposure is the inside of the work gloves. The gloves were intended to prevent skin contact with the grout. When distilled water was rinsed inside one of the gloves, the rinse water was found to contain 65 ug of acrylamide.

In the EPA study, four sewer grouting sites were investigated. However, in contrast to the NIOSH study, the primary purpose of the study was to measure dermal rather than respiratory exposure. Air monitoring was also done. At the four sites, three grouting procedures were surveyed, manhole repair (two sites), mainline repair and lateral line repair. The latter two are remote controlled operations.

Personal and area air samples were collected on a 0.8 um mixed cellulose ester filter and silica gel sampling train, using calibrated battery-operated sampling pumps at a nominal flow rate of 1.0 L/min. One area sample, except at the two manhole grouting sites, was collected inside the Mobile Reveal and Seal Unit, where the mixing tanks are located. Personal samples were collected in the breathing zone of workers during the chemical grout mixing operation and during the grout injection operation whenever manholes were entered.

Air sampling data were recorded on the Air Sampling Data Sheets (Figure 9-7). The information collected for each personal or area air sample included: employee and work site data,

Company Name	Contract Number: 68-02-3938	Date (Mo/Da/Yr)
Site ID	Work Assignment No. 47	Substance Monitored: ACRYLAMIDE

EMPLOYEE AND WORK AREA DATA

Employee Name	Work Location Description
Job Title/Work Duties	
	Weather Conditions

SAMPLING EQUIPMENT

Instrument	Model No.	Serial No.
[] Personal [] Area [] Bulk	Sample Collection Media: 0.8u MIXED CELLULOSE ESTER FILTER AND SILICA GEL TUBE	Lot No.

FIELD SAMPLING INFORMATION

Field Sample ID Number		
Start Time		
Stop Time		
Sample Duration (mins.)		
Pump Flow Rate ( )		
Sample Air Volume ( )		

Laboratory Analysis		Laboratory Results	Air Concentration	Laboratory Results	Air Concentration	Laboratory Results	Air Concentration
Analyte	Units→						
1. ACRYLAMIDE							
2.							
3.							

Signature:	Date:
Calculations Checked by:	Date:

Figure 9-7. Air Sampling Data Sheet

CALIBRATION DATA			
Calibration Method			
Volume		Resistance	
Pre Date	Post Date	Calculations	
1.	1.		
2.	2.		
3.	3.		
Signature	Signature	Average Time	Flowrate

Additional Comments, Observations, Diagrams, Data References, etc.

Figure 9-7 Cont.

sampling equipment data, sampling parameters, exposure data, calibration data and general observations (work practices, safety equipment, etc.).

Dermal contact sampling was performed using the dermal pad and hand rinse methods as described by Durham and Wolfe (1962). Observations made during a preliminary site visit to a chemical grouting operation indicated that significant dermal contact could occur on the face, neck, and forearms of workers even though they wore impervious clothing. The worker's torso, upper arms, and legs are protected by the impervious suit. When full protective clothing was used by a worker, dermal pads were placed at six body locations to assess dermal contact to the face, neck and forearms. When protective clothing was not utilized, dermal pads were placed at ten body locations to assess dermal contact to the entire body. Hand rinses were conducted using the bag techniques as first described by Durham and Wolfe (1962). Dermal contact assessments were conducted during equipment assembly operations, grout mixing operations, grout injection operations, and equipment disassembly operations.

Air monitoring results expressed as 8-hour TWA's appear in Table 9-9. The 8-hour TWA's ranged from ND to 0.12 mg/m^3, with a mean of 0.045 mg/m^3. Except for the maintenance supervisor at site number one, the values are similar to the NIOSH data for manufacturing/processing workers. However, the dermal monitoring indicates significant dermal exposure, even among workers using safety equipment. (See Tables 9-10, 9-11, 9-12, and 9-13.)

Table 9-9 Acrylamide Inhalation Exposures

Sample description	Air conc.[a] (mg/m^3)	8-h TWA[b] (mg/m^3)	OSHA PEL[c] (mg/m^3)*	ACGIH TLV[d] (mg/m^3)
Site no. 1				
Breathing zone of Maintenance Supr.	0.360	0.120	0.03	0.03
Breathing zone of Util. Worker no. 1	0.100	0.003	0.03	0.03
Site no. 2				
Breathing zone of Util. Worker no. 2	0.040	0.010	0.03	0.03
Site no. 3				
Breathing zone of Grout Foreman	0.060	0.060	0.03	0.03
Breathing zone of Laborer	0.040	0.040	0.03	0.03
Area sample near mixing tanks	0.050	0.050	0.03	0.03
Site no. 4				
Breathing zone of Utility Worker	0.008	0.008	0.03	0.03
Area sample near mixing tanks	0.080	0.070	0.03	0.03
Area sample approx. 20 ft from service van	ND[e]	ND	0.03	0.03

[a]Air concentration of acrylamide during sampling period in milligrams per meter of air.
[b]Eight-hour time weighted average exposure (TWA) assuming no exposure to acrylamide during periods not sampled.
[c]Occupational Safety and Health Administration Permissible Exposure Limit.
[d]Threshold limit value (TLV) published by the American Conference of Governmental Industrial Hygienist (1987-1988 edition).
[e]ND = none detected, the detection limit was 0.004 mg/m^3 for a 480-L air sample.
* "skin" notation

Table 9-10 Dermal Contact Estimate

[redacted]te no. 1
Manhole Sealing Operation
Maintenance Supervisor

Body region	Surface area of region[a] (cm^2)	Exposure pads used to represent body region	Surface area of pads (cm^2)	Total acrylamide found (mg)	Time of exposure (h)	Dermal contact estimate[b] (mg/h)
Head	1,090	shoulder pads (4)	400	1.76	2.9	1.6
Back of neck	115	back pads (2)	200	0.109	2.9	0.02
Front of neck	115	chest pads (2)	200	6.75	2.9	1.3
Forearms	1,290	forearm pads (4)	400	0.386	2.9	0.43
Hands	N/A	hand rinses (6)	N/A	12.1	7.4	1.7
					Total Dermal Contact Estimate =	5.0

[a]urface area of body regions based on the anatomic dimensions of the 50th ercentile man from Popendorf (1976, 1982). Diffrient et al. (1974), and NASA (1962).

[b]Dermal contact estimate (mg/h) = $\frac{A_{TOT} \times S_1}{T \times S_2}$

where A_{TOT} = total acrylamide found (mg)

S_1 = surface area of body region (cm^2)
S_2 = surface area of pads (cm^2)
T = time of exposure (h).

Table 9-11 Dermal Contact Estimate

Site no. 2
Manhole Sealing Operation
Utility Worker no. 2

Body region	Surface area of region[a] (cm^2)	Exposure pads used to represent body region	Surface area of pads (cm^2)	Total acrylamide found (mg)	Time of exposure (h)	Dermal contact estimate[b] (mg/h)
Head	1,090	shoulder pads (4)	400	0.267	3.0	0.24
Back of neck	115	back pads (2)	200	0.050	3.0	0.01
Front of neck	115	chest pads (2)	200	0.020	3.0	0.004
Forearms	1,290	forearm pads (4)	400	2.18	3.0	2.3
Hands	N/A	hand rinses (8)	N/A	0.445	7.7	0.058
					Total Dermal Contact Estimate =	2.6

[a]Surface area of body regions based on the anatomic dimensions of the 50th percentile man from Popendorf (1976, 1982), Diffrient et al. (1974), and NA[illegible] (1962).

[b]Dermal contact estimate (mg/h) = $\frac{A_{TOT} \times S_1}{T \times S_2}$

where A_{TOT} = total acrylamide found (mg)

S_1 = surface area of body region (cm^2)
S_2 = surface area of pads (cm^2)
T = time of exposure (h).

Table 9-12 Dermal Contact Estimate

Site no. 3
Mainline Operation

Body region	Surface area of region[a] (cm^2)	Exposure pads used to represent body region	Surface area of pads (cm^2)	Total acrylamide found (mg)	Time of exposure (h)	Dermal contact estimate[b] (mg/h)
Grout Foreman						
Head	1,090	shoulder pads (2)	200	0.241	9.3	0.14
Back of neck	115	back pad (1)	100	0.081	9.3	0.01
Front of neck	115	chest pad (1)	100	0.123	9.3	0.02
Forearms	1,290	forearm pads (2)	200	1.26	9.3	0.87
Hands	N/A	hand rinses (6)	N/A	7.40	10.2	0.73
					Total Dermal Contact Estimate =	1.8
[redacted]borer						
Head	1,090	shoulder pads (2)	200	0.158	9.3	0.09
Back of neck	115	back pad (1)	100	0.067	9.3	0.008
Front of neck	115	chest pad (1)	100	0.099	9.3	0.01
Forearms	1,290	forearm pads (2)	200	0.086	9.3	0.06
Hands	N/A	hand rinses (6)	N/A	4.54	10.2	0.44
					Total Dermal Contact Estimate =	0.61

[a]Surface area of body regions based on anatomic dimensions of the 50th percentile man from Popendorf (1976, 1982), Diffrient et al. (1974), and NASA (1962).

[b]Dermal contact estimate (mg/h) = $\frac{A_{TOT} \times S_1}{T \times S_2}$

where A_{TOT} = total acrylamide found (mg)

S_1 = surface area of body region (cm^2)
S_2 = surface area of pads (cm^2)
T = time of exposure (h).

Table 9-13 Dermal Contact Estimate

Site no. 4
Lateral Line Operation
Utility Worker

Body region	Surface area of region[a] (cm^2)	Exposure pads used to represent body region	Surface area of pads (cm^2)	Total acrylamide found (mg)	Time of exposure (h)	Dermal contact estimate[b] (mg/h)
Head	1,090	shoulder pads (2)	200	0.018	8.2	0.01
Back of neck	115	back pad (1)	100	0.008	8.2	0.001
Front of neck	115	chest pad (1)	100	0.006	8.2	0.001
Back	1,540	back pad (1)	100	0.008	8.2	0.02
Chest	1,540	chest pad (1)	100	0.006	8.2	0.01
Upper arms & shoulders	3,170	shld. & forearm pads (4)	400	0.062	8.2	0.06
Forearms	1,290	forearm pads (2)	200	0.044	8.2	0.03
Thighs & hips	5,210	thigh pads (2)	200	0.112	8.2	0.36
Lower legs & feet	3,820	shin pads (2)	200	0.131	8.2	0.30
Hands	N/A	hand rinses (6)	N/A	0.494	8.2	0.06

Total Dermal Contact Estimate = 0.85

[a]Surface area of body regions based on the anatomic dimensions of the 50th percentile man from Popendorf (1976, 1982), Diffrient et al. (1974), and NASA (1962).

[b]Dermal contact estimate (mg/h) $= \frac{A_{TOT} \times S_1}{T \times S_2}$

where A_{TOT} = total acrylamide found (mg)

S_1 = surface area of body region (cm^2)
S_2 = surface area of pads (cm^2)
T = time of exposure (h).

Based on previous observational site visits (which included still and video recording), EPA expected that manhole repair had potentially the highest dermal exposure. The results of the monitoring study confirm this hypothesis. The two manhole sites had total dermal contact estimates of 5.0 and 2.6 mg/hr, whereas the mainline and lateral line repair sites were 1.8, 0.61 and 0.85 mg/hr, respectively. In addition, one worker had the classic symptoms of acrylamide-induced peripheral neurotoxicity such as skin peeling. Two other workers indicated that they had experienced peeling skin on the hands.

9.4.3. Potable Water Consumption

Polyacrylamide products are used as flocculants to clarify raw water in potable water plants. Because a low level of residual acrylamide monomer is present in the polymer, treated water may contain a certain amount of acrylamide. How much depends on polymer use levels, residual monomer levels, and the removal, if any, of acrylamide in drinking water treatment.

Based on data supplied by the Synthetic Organic Chemical Manufacturers Association (SOCMA) (1986), typical monomer concentrations in drinking water are about 0.01 ppb. This is derived from 0.2 ppm (typical polymer use level) X 50 ppm (typical residual monomer in polymer) = 0.01 ppb. Assuming the consumption of two liters of water per day, monomer consumption would be 2.8×10^{-7} mg/kg/day for a 70 kg person. The maximum concentration is 0.5 ppb, assuming the maximum permitted

Table 9-14. Acrylamide Concentrations in Sugar

Type of Sugar	Polymer Conc. ppm	Monomer Conc. ppm	Resultant Monomer Conc.* ppb
Granulated	4	50	0.012**
Soft sugars	4	50	0.24-0.48**
Molasses	4	50	42.4**
Cane/beet juice	3-4	50	0.15-0.2***
Cane/beet liquor	10	50	0.5***
Corn sweeteners	8	50	0.4***

* Assumes no degradation during sugar processing or losses during clarification/filtration.
** Assumes monomer partitions with water.
*** Assumes monomer partitions with polymer.

Table from SOCMA (1986).

9.4.5. Other

No data are available on the extent and magnitude of acrylamide exposure for persons using the various polyacrylamide products discussed in section 9.2.2. However, based on the nature of the uses of these products, the fact that acrylamide is present as an impurity, they are added to process streams either remotely or infrequently by bucket, and that the polymers are added in aqueous solutions indicates that the exposure potential would be low. The laboratory use of acrylamide for gel electrophoresis presents the potential for significant dermal exposure whenever gloves are not worn in handling the monomer or gel.

10. RISK ESTIMATION

The determination of individual risk can be made through the use of epidemiologic or animal studies. Epidemiologic studies suitable for low-dose quantitative risk extrapolations are rarely available, and are not available in the case of acrylamide. For acrylamide, cancer, neurotoxic, and reproductive risks were estimated through the use of animal data. This necessitates extrapolation from high to low doses because, typically, test animals are exposed to concentrations higher than those expected to be experienced by humans. These extrapolations are carried out by fitting mathematical models to the observed animal data for cancer risk estimation and by calculating margins of exposure from NOEL and LOEL data for noncancer health hazards.

10.1. Noncancer Health Risks

The hazard section of the risk assessment identified four noncancer effects of acrylamide: neurotoxic, reproductive, developmental, and genotoxic effects. The data bases for these effects are comprised primarily of animal data; only in the case of neurotoxic effects are human data available. The strength of the associations vary considerably, with neurotoxicity being the strongest and developmental effects the weakest. To evaluate the risk potential for acrylamide's noncancer effects, each effect is examined here in turn and where possible, risk estimates are generated. The traditional method of assessing the risk

presented by noncarcinogens is to calculate an "acceptable daily intake" or ADI. The ADI is calculated by applying "safety factors" in multiples of ten to either NOEL's or LOEL's derived mainly from animal studies. For instance, if the NOEL in a 90-day feeding study were 5 mg/kg/d, the ADI would be 0.05 mg/kg/d. This is derived by dividing the NOEL by a safety factor of 100 (10 for less than a chronic study and an additional 10 to account for animal to human extrapolation). This would be considered a "safe" level of exposure. This approach is an assumption that there is a threshold dose below which no toxic effects occur. If there are any residual risks at the ADI, they are at most trivial and of no public health significance (Rodricks and Taylor, 1983).

Unfortunately, the term "safety factor" suggests, perhaps inadvertently, the notion of absolute safety, i.e., absence of risk. While there is a conceptual basis for believing in the existence of a threshold and "absolute safety" associated with certain chemicals, in the majority of cases a firm experimental basis for this notion does not exist.

As a consequence of the use of the term "safety factor", the ADI is viewed by many as an "acceptable" level of exposure, and, by inference, any exposure greater than the ADI is seen as "unacceptable". This strict demarcation between what is "acceptable" and what is "unacceptable" is contrary to the views of most toxicologists, who typically interpret the ADI as a relatively crude estimate of a level of chronic exposure which is not likely to result in adverse effects to humans. The ADI is

generally viewed as a "soft" estimate, whose bounds of uncertainty can span an order of magnitude. That is, within reasonable limits, while exposures somewhat higher than the ADI are associated with increased probability of adverse effects, that probability is not a certainty. Similarly, while the ADI is seen as a level at which the probability of adverse effects is low, the absence of risk to all people cannot be assured at this level.

In response to the conceptual problems associated with ADIs and safety factors, the concept of the "reference doses (RfD)" was developed (EPA, 1986). The RfD is a benchmark dose operationally derived by consistent application of generally order of magnitude uncertainty factors (UFs) that reflect various types of data used to estimate RfDs (for example, a valid chronic human NOAEL normally is divided by an UF of 10-fold) and an additional modifying factor (MF), which is based on a professional judgement of the entire data base of the chemical. The RfD is determined by use of the following equation:

$$RfD = NOAEL/(UF \times MF)$$

In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude or greater) of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is appropriately expressed in units of mg/kg/day.

The RfD is useful as a reference point for gauging the

potential effects of other doses. Usually, doses which are less than the RfD are not likely to be of regulatory concern. However, as the frequency of exposures exceeding the RfD increases, and as the size of the excess increases, the probability increases that adverse effects may be observed in a human population.

In the risk characterization process, a comparison can be made between the RfD if one has been established and the estimated (calculated or measured) exposure dose, which should consider exposure by all sources and routes of exposure.

An alternative measure that is useful and which will be used here in addition to an RfD comparison is the "margin of exposure (MOE)", which is the magnitude by which the NOAEL of the critical toxic effect exceeds the estimated exposure dose where both are expressed in the same units:

MOE = NOAEL (experimental dose) / (human dose)

For instance, if a NOEL of 0.3 mg/kg/day were used and the human exposure was predicted or measured at 0.1 mg/kg/day, then the MOE would be 3. The lower the MOE the higher the concern. However, in each case, consideration of the MOE derived must be tempered by consideration of the quality of the data base used.

10.1.1. Neurotoxicity

While there are a variety of effects on the nervous system from chronic acrylamide exposures, those that are most serious, widely studied, and appear most prominent are the effects on

motor systems in peripheral nerves and the spinal cord. Because the human data are primarily limited to case reports of unquantified exposure levels, a quantitative risk assessment for neurotoxicity must rely on the best available animal data. In general, the available studies reveal no peculiarities in the sensitivity of exposed animal groups, although most studies are limited by design and group size in their ability to elucidate such issues. Most of the animal species tested for these effects showed a sensitivity in the same general exposure range, which provides some confidence in making interspecies extrapolations.

The best animal studies for evaluating chronic acrylamide exposures are summarized in Table 5-2 of section 5.3.3. The lowest observed effect levels (LOEL's) for three species (rats, cats, and monkeys) are reported as 1 mg/kg/day. For cats and rats, effects at this level were seen after only 3 to 4 months of exposure. Thus, 90 days or more of exposure to 1 mg/kg/day is associated with neuropathological effects. This is generally consistent with the one quantified human exposure report (Igisu et al., 1975), where dramatic effects were observed at about 10 mg/kg/day after less than 1 month of exposure.

The no observed effect levels (NOEL's) range from 0.2 to 2.0 mg/kg/day from the best available data. For each species tested, however, the NOEL's are slightly different. For rats, 0.2 mg/kg/day for 3 months is the best NOEL (from electron microscopy study). Using light microscopy, however, a 2-year NOEL of 0.5 mg/kg/day was observed. Although a 2-year study, it was not

judged the best because the NOEL was found with light microscopy, a fairly insensitive measure of structural integrity. For cats the NOEL is 0.3 mg/kg/day for 1 year and for monkeys the NOEL is less than 1.0 mg/kg/day for 1.5 years.

Margins of exposure (MOE) were calculated based on the lowest, best NOEL of 0.2 mg/kg/day from the Burek et al. (1980) study (see Table 10-1). A similar NOEL of 0.5 mg/kg/day was observed in the Johnson et al. (1986) rat study. The MOE's are for chronic workplace or dietary exposure.

As is evident from Table 10-1, soil grouting workers have very low MOE's. This may account for the observation of overt signs of acrylamide neurotoxicity in one of the nine workers interviewed during the EPA-sponsored field study (See section 9.5.2.). Two other workers indicated that they had experienced peeling skin on the hands. Even more important is the fact that the MOE's using the lowest LOEL of 1.0 mg/kg/day, which has been reported for rats, cats, and monkeys, range from 6 to 43. For comparison, an RfD based on a LOEL would be derived by dividing the LOEL by an uncertainty factor of 1,000. The oral RfD for chronic acrylamide exposure is 0.0002 mg/kg/day which is significantly below the exposures reported for soil grouting workers.

The MOE's for the manufacturing/processing groups range from 29 to 182. Drinking water MOE's are greater than 1,000.

TABLE 10-1. MARGINS OF EXPOSURE (MOE) FOR CHRONIC ACRYLAMIDE EXPOSURE -- NEUROTOXICITY

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Exposure in mg/kg/d	MOE for NOEL of 0.2 mg/kg
OSHA Std.	N/A	0.03 mg/m^3 (skin)	4.3×10^{-2}	5
Cyanamid[a]	57	monomer prod. 0.045 mg/m^3	6.4×10^{-3}	31
		polymer prod. 0.060 mg/m^3	8.6×10^{-3}	23
Nalco[a]	26	monomer/ polymer prod. 0.005 mg/m^3	7.1×10^{-4}	282
Dow[a]	55	monomer prod. 0.049 mg/m^3	7.0×10^{-3}	29
		polymer prod. 0.007 mg/m^3	1.0×10^{-3}	200
Soil Grouting[a]	1800	0.005 mg/m^3	7.1×10^{-4}	282
Soil Grouting[a]	site 1	0.12 g/m^3 / 1.25 mg/hr	1.6×10^{-1}	1
Soil Grouting	site 2	0.01 mg/m^3 / 0.65 mg/hr	7.6×10^{-2}	3

TABLE 10-1 -- CONTINUED

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Exposure in mg/kg/d	MOE for NOEL of 0.2 mg/kg
Soil		0.06 mg/m^3		
Grouting	site 3a	----------	6.0×10^{-2}	3
		0.45 mg/hr		
Soil		0.04 mg/m^3		
Grouting	site 3b	----------	2.3×10^{-2}	9
		0.15 mg/hr		
Soil		0.008 mg/m^3		
Grouting	site 4	----------	2.5×10^{-2}	8
		0.21 mg/hr		
Drinking Water[c]	3×10^7 max. possible		0.5 ppb maximum**	>1,000
			$\frac{1.4 \times 10^{-5}}{0.01 \text{ ppb typical}}$	----
			2.8×10^{-7}	>>1,000

* For inhalation and ingestion 100% absorption is assumed. For dermal absorption, the mg/hr estimates in Tables 9-10 to 9-13 were multiplied by 0.25 to account for the difference in absorption by this route.
** Concentration in water treatment plant.

[a] NIOSH (1985).
[b] EPA (1987).
[c] SOCMA (1986).

10.1.2. Reproductive Effects

The reproductive effects of acrylamide have been observed in several animal studies (Section 6). A dominant lethal effect and decreased copulatory performance have been observed in rats. In one assay, rat testosterone levels were depressed, but the functional significance of this finding is uncertain. In the mouse, decreased fertility was observed following exposure of male mice to acrylamide and an increased resorption rate resulted from the exposure of male or female mice. Degeneration of testicular epithelial tissue and a dominant lethal effect have also been observed in other mouse studies.

The available data indicate that acrylamide can act directly on the reproductive system, rather than indirectly through stress or other systemic effects such as neurotoxicity. Therefore, concern for the reproductive toxicity potential is heightened. In other data reviewed in the rat and the mouse, neurotoxicity occurred in each case, while reproductive toxicity was sometimes absent. It appears then, that while acrylamide can exert a direct effect on the testes, the weight of evidence for effect levels and probability of occurrence for acrylamide induced neurotoxicity is much higher.

The LOEL and NOEL for reproductive effects, as indicated by the available data (Table 6-2 in section 6.1.), are 8.8 and 4.2 mg/kg/day, respectively. As Table 10-2 indicates, all groups have MOE's an order of magnitude or more greater than those for neurotoxicity (chronic exposure).

TABLE 10-2. MARGINS OF EXPOSURE (MOE) FOR ACRYLAMIDE BASED REPRODUCTIVE EFFECTS

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Exposure in mg/kg/d	MOE for NOEL of 4.2 mg/kg	
OSHA Std.	N/A	0.03 mg/m^3(skin)	4.3×10^{-2}	98	3
Cyanamid[a]	57	monomer prod. 0.045 mg/m^3	6.4×10^{-3}	656	18
		polymer prod. 0.060 mg/m^3	8.6×10^{-3}	488	13
Nalco[a]	26	monomer/ polymer prod. 0.005 mg/m^3	7.1×10^{-4}	595	160
Dow[a]	55	monomer prod. 0.049 mg/m^3	7.0×10^{-3}	600	16
		polymer prod. 0.007 mg/m^3	1.0×10^{-3}	4,200	112
Soil Grouting[a]	1800	0.005 mg/m^3	7.1×10^{-4}	5,915	160
Soil Grouting[b]	site 1	0.12 g/m^3 / 1.25 mg/hr	1.6 X 10-1	26	0.7
Soil Grouting	site 2	0.01 mg/m^3 / 0.65 mg/hr	7.6×10^{-2}	55	2

TABLE 10-2 -- CONTINUED

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Exposure in mg/kg/d	MOE for NOEL of 4.2 mg/kg	
Soil Grouting	site 3a	$0.06\ mg/m^3$ / 0.45 mg/hr	6.0×10^{-2}	70	2
Soil Grouting	site 3b	$0.04\ mg/m^3$ / 0.15 mg/hr	2.3×10^{-2}	183	5
Soil Grouting	site 4	$0.008\ mg/m^3$ / 0.21 mg/hr	2.5×10^{-2}	168	5
Drinking Water[c]	3×10^7 max. possible		0.5 ppb maximum**	>1,000	
			1.4×10^{-5}	>100,000	
			0.01 ppb typical	>>1,000	
			2.8×10^{-7}	>100,000	

* For inhalation and ingestion 100% absorption is assumed. For dermal absorption, the mg/hr estimates in Tables 9-10 to 9-13 were multiplied by 0.25 to account for the difference in absorption by this route.
** Concentration in water treatment plant.

[a] NIOSH (1985)
[b] EPA (1987)
[c] SOCMA (1986)

10.1.3. Developmental Effects

Acrylamide has been shown to produce developmental and postnatal effects in mouse and rat offspring following administration to pregnant dams. On a physiological level, there is evidence that acrylamide produces neurotoxic effects (tibial and optic nerve degeneration) in the neonates at levels that are not toxic to the dam. In addition, on a biochemical level, acrylamide causes changes in dopamine levels and intestinal enzyme levels in the fetus at dose levels where no maternal toxicity is apparent, but the toxicological significance of these biochemical changes is not clear from the available data. There is a basis for concern for the conceptus following maternal exposure during gestation since these data show that acrylamide can have a direct effect on the conceptus. However, the specific doses at which neurotoxic effects were observed in rat offspring was not reported in the study. Consequently, numerical risk estimates cannot be calculated for these neurotoxic effects. For the biochemical effects, the data indicate a lowest observed effects level (LOEL) of 20 mg/kg/day. Since this was the lowest dose level tested in the study, a no observed effect level (NOEL) was not established (Table 6-1 in section 6.1). However, because the toxicological significance of these biochemical changes is not apparent, risk of functional deficits cannot be estimated.

10.1.4. Genotoxic Effects

The genotoxicity data on acrylamide provide a basis to support the carcinogenicity hazard identification and to support heritable mutation as a separate endpoint of concern for acrylamide exposures.

The guidelines for mutagenicity risk assessment address evaluation of the potential genetic risk associated with human exposure to chemicals with effects such as those of acrylamide. The body of evidence suggests that acrylamide may induce alterations in the genome of germinal cells. These data provide a strong weight of evidence bearing on the potential for human germ-cell mutagenicity and its heritability. The strength of a human germ-cell mutagenicity concern would only be further strengthened by direct human evidence (the highest level of evidence for human mutagenicity).

The existing mutagenicity testing results for acrylamide demonstrate that it induces chromosome breaks but not point mutations. In addition, germ cell cytogenetics and in vivo germ cell test results in males of two species adequately demonstrate that acrylamide can reach cellular targets in mammalian germ cells and produce chromosomal aberrations that can be transmitted to the next reproductive generation. However, the data do not allow the development of reliable quantitative estimates of heritable risk. Therefore, the remainder of this section will simply illustrate ways to estimate the potential magnitude of such risks. Undue emphasis should not be placed on the

quantitative risk estimates of that risk. The ensuing discussion will examine the effects of acrylamide in inducing dominant lethal effects which are noted in the offspring of exposed parents and chromosomal translocations, a proportion which have deleterious heritable consequences.

A quantitative estimate of risk for a biological effect is dependent upon an estimate of the shape of the dose-response curve for the effect under review and the magnitude and nature of anticipated exposure. In combination these two considerations give a measure of the incidence or frequency of the given endpoint. In quantitating risk from some heritable effects a second factor must be considered; that is, the proportion of mutations that may be manifest as adverse health effects in the recipients of the mutations.

From a theoretical standpoint dominant lethal mutations may be conceived as the result of a single break in a chromosome. This leaves an acentric fragment which is not able to move properly in cell division. The resulting conceptus is unable to survive embryonic and fetal life and dies in utero, thus the term, dominant lethal. If each chemical-chromosome interaction could result in a break, then the expected dose-response curve would be linear, at least at low doses. In practice, however, we do not know how many interactions are required to induce a chromosomal break, but it seems reasonable that a linear dose-response relationship may apply under certain circumstances and, at least, could represent a reasonable upper bound on the risk.

For translocations that are passed from treated parent to offspring, two different chromosomes would need to be broken and their corresponding arms recombined. Thus, theoretically at least two independent chemical-chromosome interactions would be necessary. Such a requirement would suggest that dose-response curves would be curvilinear upward.

Evaluation of the existing data on acrylamide does not allow one to make definitive judgements about the shape of the dose-response curve. First, the molecular mechanisms by which acrylamide induces chromosome breaks are essentially unknown. Second, because the doses in the mouse heritable translocation test are so close together (40 & 50 mg/kg/d) it does not allow one to characterize the underlying dose-response relationship. Third, there are gaps in the data base for all the germ cell tests conducted on acrylamide. These include a lack of low and multiple dose testing, inability to discern dose-rate interrelationships, lack of reproductive performance data on individual treated males, and lack of standardized protocols. These shortcomings limit the ability to characterize the heritable risks for this agent. Thus, instead of an in-depth analysis of the risk, only rough estimates will be developed as an illustration of how one may proceed to conduct these analyses.

It is evident from the mouse germ cell data that the mutagenic response to acrylamide exposure differs significantly as a function of the germ cell stage. Like a number of other chemical substances that produce chromosomal breakage in male

germ cells (e.g., ethylene oxide, ethylmethanesulfonate), it appears that acrylamide exerts its major effect upon post-meiotic stages of sperm development (i.e., late spermatid and sperm). Thus, it is the germ cells in about the last two weeks of their development that are at risk for mutation. This implies that although there may be some risk of mutation shortly after exposure to acrylamide, that risk may decrease greatly following longer intervals of time as the cells at risk fully mature and disappear from the male reproductive tract.

In an attempt to make a rough estimate of potential risk from exposure to acrylamide, a number of assumptions were made which will be applied to both dominant lethal effects and heritable translocations.

a) Mouse germ cells are at risk during the last two weeks of development.

b) The post-meiotic sensitive period is the same length in humans as in the mouse.

c) The mutagenic response of humans and animals is comparable on a body weight basis.

d) The relevant exposure is the accumulated total received over a 2-week period.

Due to the shortcomings in the design and reporting of the data sets on acrylamide, only point estimates of potential mutagenic risk are presented (to the nearest order of magnitude); confidence intervals on the models are not included.

Four dominant lethal studies were examined: two in mice and two in rats. Each of the mouse studies (Shelby et al., 1986; 1987) involved a single dose level of acrylamide and a control. The two rat studies (Smith et al., 1986; Nalco, 1987) used several dosages. A simple linear extrapolation was performed from the lowest dose showing an effect to the origin. When dominant lethals were recorded as function of post-treatment time (Shelby et al., 1986), the average proportion of dominant lethals over a two-week period (approximate) was used for the linear extrapolation. Exposure to treated animals was assumed to be the total received over a two-week period (e.g., 5 mg/kg/d x 14d = 70 mg/kg in drinking water (Nalco, 1987)). Slope factors (risk per unit exposure) were calculated for each study; a similar estimate was found for all studies (0.001 $(mg/kg)^{-1}$), except the Smith et al. study which was about an order of magnitude lower.

Estimates of dominant lethal risk were made, assuming that exposures were equivalent to the soil grouting workers (table 10-5) that had been monitored. If daily dermal and inhalation exposures range from about 0.02 to 0.16 mg/kg, then two work weeks of exposure would vary between about 0.20 and 1.6 mg/kg. Thus, accumulated excess risk that might accrue over a 2 week period following exposure might then range from 10^{-4} to 10^{-3}, respectively.

An analogous calculation can be made using the mouse heritable translocation data, assuming a linear dose response as

suggested by Bishop and Kodell (1980). A slope was calculated using the 200 mg/kg total exposure (40 mg/kg/d for 5d); it was the same as that calculated for the dominant lethal studies ($0.001\ (mg/kg)^{-1}$). Thus, extrapolation to the level of exposure reported for the human grouting workers leads to risks in the range of 10^{-4} to 10^{-3}. Recognizing that heritable translocations may not behave in a linear fashion with dose, other models were applied to all dose groups in the mouse study to extrapolate to the human exposure situation. Using a multistage model with two stages, point estimates of the risks were in the range of 10^{-7} to 10^{-5}. A third mathematical construct, the quadratic, assumes two chemical-cell interactions for an effect; this model predicted point estimates of the risks as 10^{-7} to 10^{-5}. Given the theoretical requirement of at least two breaks to form a translocation, it would appear that a linear extrapolation might overestimate risks, whereas the multistage and quadratic approaches are less conservative.

To evaluate the risk estimates further, it is important to recognize that balanced translocations per se are not expected to be associated with health consequences. It is only when there is abnormal segregation out of the heterozygote to form gametes with unbalanced genomes that adversity is expected. From the little information known about human carriers of balanced translocations, it would appear that the likelihood of adversity and the nature of the effects on reproduction and offspring survival varies with the type of translocation. Carriers of some

translocations show about 25 percent changes relative to non-carriers: decreases in live births, increases in fetal deaths, and reductions in reproductive fitness (Morton et al., 1975; Jacobs et al., 1975). These effects in humans are like those noted in mice with translocations (Generoso et al., 1980).

The present estimates of heritable chromosomal risk indicate that acrylamide exposures associated with a certain occupational setting (i.e., soil grouting) may be associated with dominant lethal effects at risk levels about two orders of magnitude below estimated lifetime excess cancer risks. (See section 10.2. Table 10-4.) The corresponding risk for heritable translocations may be as high as the dominant lethal risks but may be orders of magnitude lower than that. Even though these risk estimates for heritable effects are only preliminary, they should not be totally ignored.

10.2. Quantitative Cancer Risk Assessment

Since risks at low exposure levels cannot be measured directly either by experiments in animals or by epidemiologic studies, a number of mathematical models have been developed to extrapolate from high to low doses. The Office of Science and Technology Policy (OSTP) published principles on model selection which states that "No single mathematical procedure is recognized as the most appropriate for low-dose extrapolation in carcinogenesis. When relevant biological evidence on mechanism of action exists, the models or procedures employed should be

consistent with the evidence. When data and information are limited, however, and when much uncertainty exists regarding the mechanism of carcinogenic action, models or procedures which incorporate low-dose linearity are preferred when compatible with the limited information." Data relevant to selecting a model for cancer risk extrapolation associated with exposure to acrylamide were reviewed; much of the biological information supports a direct relationship between exposure and carcinogenicity. (See section 10.2.2.) However, other mechanistic data are lacking. Thus little information was available to propose a non-linear extrapolation model to estimate the risks of acrylamide. Therefore, in keeping with the OSTP guidance and EPA's Guidelines for Carcinogen Risk Assessment, the assessment employed a linear model (i.e., linearized multistage procedure).

Data from the Johnson et al (1986) study were used to estimate risk from acrylamide exposure. The EPA guidelines for cancer risk assessment recommend pooling tumor incidence data for purposes of risk assessment, since risk numbers derived from site-specific tumor incidence data may not be predictive of (and may in fact underestimate) "whole-body" risks that are determined using the pooled individual animal data. The dose-response curves for each sex based on the pooled tumor incidence (benign and malignant) data compromise the data sets of choice for risk assessment. The most sensitive sex will be chosen to represent possible human risk. Further, a second pool was created for each sex to allow consideration of the contribution of malignancies

alone to the overall risk estimates. Table 10-3 summarizes the pooled tumor incidence data. The dose levels used in the extrapolation; 0, 0.01, 0.1, 0.5, and 2 mg/kg/day, had been previously derived by adjustment for varying water consumptions across time in the study and across dose levels.

Tumors at a particular site were added into the pool only when the tumor site had statistically significantly increased incidence at least at the high dose level (treated vs. control). The first pool contains males having tumors of the testes, thyroid, or adrenal gland. For the adrenal gland, only the (benign) adenomas were considered since neither malignant alone, nor adenoma and adenocarcinoma combined, were significantly elevated. The second male pool contains testes mesothelioma only. The third pool contains females having tumors of the thyroid (combined), mammary gland (combined), CNS (tumors only, no "proliferations"), uterus (adenocarcinoma), and oral (combined). The fourth pool contains females having malignant tumors of the thyroid, mammary gland, CNS, and uterus. Tumors of the clitoral gland in females are not included in the pooled tumor incidence data because only a very low number of tissues were examined in each dose group.

A trans-species conversion for risk was carried out. For example, if male humans weigh approximately 70,000g and female rats weigh approximately 200g then, by the method of Mantel and Schneiderman, whereby body surface area is a surrogate for

*Changes to be made in final R.A. and brought out in public comments (See Response 4.)

Table 10-3. Animal Test Data Sets from Dow Acrylamide Study Used for Extrapolation.

Male Rats: Number of Animals with Tumors -- Testes, Thyroid, and Adrenal

Dose (mg/kg/day)	Number Responding	Number at Risk
0.0	7	59
0.01	8	52
0.1	13	57
0.5	14	57
2.0	22	54

Male Rats: Number of Animals with Malignant Tumors -- Testes

Dose (mg/kg/day)	Number Responding	Number at Risk
0.0	3	57
0.01	0	49
0.1	7	57
0.5	11	53
2.0	10	52

Female Rats: Number of Animals with Tumors -- Thyroid, Mammary, CNS, Oral, and Uterus

Dose (mg/kg/day)	Number Responding	Number at Risk
0.0	13	60
0.01	18	60
0.1	14	60
0.5	21	60
2.0	44	60

Female Rats: Number of Animals with Malignant Tumors -- Mammary, Thyroid, CNS, and Uterus

Dose (mg/kg/day)	Number Responding	Number at Risk
0.0	5	60
0.01	5	60
0.1	3	60
0.5	2	60
2.0	20	60

TABLE 10-4. SUMMARY OF ACRYLAMIDE CANCER RISK ESTIMATES

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Conc. In PPB	LADE** mg/kg/day	Cancer Risk MLE (upper-bound)
OSHA Std.	N/A	0.03 mg/m^3(skin)		1.7×10^{-2}	2×10^{-2} (8×10^{-2})
Cyanamid[a]	57	monomer prod. 0.045 mg/m^3		2.4×10^{-3}	$3X10^{-3}$ ($1X10^{-2}$)
		polymer prod. 0.060 mg/m^3		3.2×10^{-3}	$4X10^{-3}$ ($1X10^{-2}$)
Nalco[a]	26	monomer/ polymer prod. 0.005 mg/m^3		2.7×10^{-4}	$3X10^{-4}$ ($1X10^{-3}$)
Dow[a]	55	monomer prod. 0.049 mg/m^3		2.6×10^{-3}	$3X10^{-3}$ ($1X10^{-2}$)
		polymer prod. 0.007 mg/m^3		3.8×10^{-4}	$4X10^{-4}$ ($2X10^{-3}$)
Soil Grouting[a]	1800	0.005 mg/m^3		2.7×10^{-4}	$3X10^{-4}$ ($1X10^{-3}$)

TABLE 10-4 -- CONTINUED

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Conc. In PPB	LADE** mg/kg/day	Cancer Risk MLE (upper-bound)
Soil Grouting[b]	site 1	0.12 mg/m^3 ---------- 1.25 mg/hr		6.3×10^{-2}	$8X10^{-2}$ ($3X10^{-1}$)
Soil Grouting	site 2	0.01 mg/m^3 ---------- 0.65 mg/hr		3.0×10^{-2}	$4X10^{-2}$ ($1X10^{-1}$)
Soil Grouting	site 3a	0.06 mg/m^3 ---------- 0.45 mg/hr		2.4×10^{-2}	$3X10^{-2}$ ($1X10^{-1}$)
Soil Grouting	site 3b	0.04 mg/m^3 ---------- 0.15 mg/hr		9.0×10^{-3}	$1X10^{-2}$ ($4X10^{-2}$)
Soil Grouting	site 4	0.008 mg/m^3 ---------- 0.21 mg/hr		9.8×10^{-3}	$1X10^{-2}$ ($4X10^{-2}$)

TABLE 10-4 -- CONTINUED

Exposure Category/ Company	Number Exposed	Air Conc. in mg/m^3--8 hr TWA Dermal Exp. in mg/hr*	Conc. In PPB	LADE** mg/kg/day	Cancer Risk MLE (upper-bound)
Drinking Water[c]	3 X 10^7 max. possible		0.5	1.4 X 10^{-5}	4X10^{-5} (6X10^{-5})
			0.01[b]	2.8 X 10^{-7}	9X10^{-7} (1X10^{-6})
Unit Risk				1.0 X 10^{-3}	(4.5X10^{-3})

* For inhalation and ingestion 100% absorption is assumed. For dermal absorption the mg/hr contact estimates in Tables 9-10 to 9-13 were multiplied by 0.25 to account for the difference in absorption by this route.
**LADE = Lifetime Average Daily Exposure = based on 40 years occupational exposure and 70 years consumer exposure.
*** Maximum Likelihood and upper bound estimates from linearized multistage model. Concentration in water treatment plant.

[a] NIOSH (1985).
[b] EPA (1987).
[c] SOCMA (1986).

Resultant risk estimates were multiplied by 7.05 to account for metabolic differences between rats and man as discussed in section 10.2. No adjustment was made to the risk estimates due to different routes of exposure because the available studies show that the pharmacokinetics and tissue distribution of acrylamide were not significantly affected by the dose administered, by the route of administration, or by giving the chemical over consecutive days. (See section 10.2.2. below for further discussion.) Dermal exposure estimates were adjusted before risk estimation to reflect lower absorption (approximately 25 percent) by the dermal route. One hundred percent absorption was assumed for oral and inhalation exposures which is supported by the laboratory data. For example, the lifetime average daily exposure (LADE) of 6.3×10^{-2} mg/kg/day for soil grouting, site 1, maintenance supervisor was calculated as follows. The inhalation contribution is 0.12 mg/m^3. This level was multiplied by (1.25 m^3/hr x 8 hours) then divided by 70 kg giving 0.017 mg/kg. The contribution by dermal exposure was 5.0 mg/hour. This is multiplied by 8 hours - 70 kg and by 0.25 (the absorption factor) to get 0.143 mg/kg. Combining by addition to get a total exposure of 0.16 mg/kg, this is further adjusted for lifetime exposure for use as a LADE in risk calculation by substitution into the estimation procedure. This adjustment calls for multiplication by 5/7 days/week, 50/52 weeks/year and 40/70 working years/lifetime or 0.392. Thus the LADE is 0.161 mg/kg x $0.392 = 6.3 \times 10^{-2}$ mg/kg/day.

The highest estimated upper-bound excess risks are to persons engaged in sewer repair or grouting operations where these extra risks range from 10^{-3} to 10^{-1}. Adjusting for less than 40 hours per week and exposures other than 50 weeks per year leaves the risk in the 10^{-3} to 10^{-2} range. For instance, if one assumes that the maintenance supervisor from site 1 only grouts 20 hours per week for 4 months per year, the estimated risk is still in the 10^{-2} range ($0.5 \times 0.33 \times 3 \times 10^{-1} = 5 \times 10^{-2}$). Such assumptions reflect that municipal grouting operations do not occur year-round and that grouting is not performed daily.

The manufacturing/processing group has upper-bound risks in the 10^{-3} to 10^{-2} range. Persons exposed to acrylamide via drinking water have upper-bound risks of 10^{-6} to 10^{-5}.

As discussed in section 10.2. above, two pooled tumor data sets were examined with the female rats all tumors being selected to represent potential human risk. The data set of male rats all tumors produced upper-bound risk estimates about an order of magnitude lower for all exposure groups. However, two other data sets were defined, males and females with malignant tumors, to allow consideration of the contribution of malignancies to the risk estimates. For male rats, all tumors, the malignant mesothelioma represents about 75 percent of the risk, whereas for female rats, all tumors, the malignancies represent about 30 percent.

10.2.2. Pharmacokinetics and Acrylamide Cancer Risk Assessment

The information on pharmacokinetics of acrylamide indicates that the applied dose is probably directly proportional to internal effective dose over a range of tested dosing conditions. Thus, human risk estimates calculated on the basis of applied dose should stand without modification at this time.

Acrylamide has none of the properties that suggest major non-linearities between applied dose and internal dose. It is rapidly and completely absorbed by the gut. It is quite uniformly distributed among tissues and, being quite polar, does not tend to accumulate in adipose tissue. It is rapidly cleared by metabolism. The proportionality of tissue accumulation of radioactive label to applied dose holds over a range of tested dosing rates.

Although metabolism is primarily by conjugation with glutathione, a number of metabolites are produced, and there is some indication that mixed-function oxidase metabolism may be involved. One cannot rule out the possibility that, although total metabolism is proportional to applied dose, the mix of various pathways may change from low to high dose, which may affect the consequent toxicity of a given amount of compound. Current data do not suggest this problem, however. The foregoing points argue that the method we used for high-to-low dose extrapolation is probably a reasonable means of analysis.

Consequently, currently available information provides no indication of nonlinearities between applied and internal dose

that would substantially alter estimates of human risk. On the contrary, it seems internal dose ought to vary in direct proportion to the rate of external application.

10.2.3. Uncertainty in Cancer Risk Estimates

In the absence of significant human data, animal data are relied upon to estimate potential human cancer risks. This introduces a number of areas of uncertainty.

First, does the finding of cancer in laboratory animals signify a similar potential in humans? And if so, are the risks predicted from animal data realistic in light of the human experience with the chemical? Does the nature of the animal data and its treatment in the risk assessment introduce additional uncertainty? Finally, do the exposure data fairly represent the magnitude and duration of exposure?

10.2.3.1. Cancer Potential in Humans

In the absence of information to the contrary, it is assumed that the appearance of cancer in laboratory animals indicates potential carcinogenicity in humans exposed to the substance. Except for two epidemiologic studies, only animal data on acrylamide carcinogenicity are available. Although one human study does show an excess of lung cancer in the exposed group, this evidence was judged to be inadequate under EPA's Cancer Risk Assessment Guidelines. The animal data do not indicate any unique susceptibility or mode of action in the species tested,

consequently it must be assumed that humans can be equally susceptible to the carcinogenic effects of acrylamide.

10.2.3.2. Uncertainty Due to Risk Estimation

Two of many factors can influence risk estimation; 1) the nature of the dose-response relationship and 2) the choice of model for risk estimation. Often the shape of the dose-response relationship, such as whether it is concave or convex, can affect the risk estimation process and increase the level of uncertainty. Also, the type of model chosen is important. For instance, models that assume a linear response at low doses are often more conservative than tolerance distribution models.

Because the two tumor data sets, males and females with tumors, are only slightly concave, the difference between the MLE's and upper-bound estimates is small. For both the data sets the difference is about a factor of two. While such a small difference does not in itself indicate that upper-bound estimates are close to or represent the true risk, it does indicate that the data are consistent with a linear response.

To test the sensitivity of the linearized multistage model, other models were used to estimate risks from acrylamide exposure. For this analysis a trans-species conversion factor of 5.85 was used. The models used were the independent and additive background probit, logit, Weibull, and gamma multi-hit. (See Appendix A for a discussion of these models.) Tables 10-5 to 10-10 present the results of this exercise. The four data sets used

Table 10-5. Males with Malignant Tumors -- Additive Background Models

Exposure LADE (mg/kg/day)	Model*	MLE	UB or UCL
8.2×10^{-3}	L.M.**	5×10^{-3}	9×10^{-3}
	A.P.	7×10^{-2}	3×10^{-1}
	A.L.	8×10^{-2}	3×10^{-1}
	A.W.	8×10^{-2}	3×10^{-1}
	A.G.	8×10^{-2}	3×10^{-1}
1.4×10^{-5}	L.M.**	8×10^{-6}	2×10^{-5}
	A.P.	2×10^{-4}	8×10^{-4}
	A.L.	2×10^{-4}	1×10^{-3}
	A.W.	2×10^{-4}	1×10^{-3}
	A.G.	2×10^{-4}	1×10^{-3}

* Linearized Multistage, and Additive Background Probit, Logit, Weibull, Gamma Multi-hit Models.
** For comparison.

Table 10-6. Males with Malignant Tumors -- Independent Background Models

Exposure LADE mg/kg/day	Model*	MLE	UB or UCL
8.2×10^{-3}	L.M.**	5×10^{-3}	9×10^{-3}
	I.P.	9×10^{-2}	3×10^{-1}
	I.L.	1×10^{-1}	4×10^{-1}
	I.W.	1×10^{-1}	4×10^{-1}
	I.G.	1×10^{-1}	4×10^{-1}
1.4×10^{-5}	L.M.**	2×10^{-6}	2×10^{-5}
	I.P.	6×10^{-4}	6×10^{-3}
	I.L.	7×10^{-3}	4×10^{-2}
	I.W.	8×10^{-3}	5×10^{-2}
	I.G.	1×10^{-2}	5×10^{-2}

* Linearized Multistage, and Independent Background Probit, Logit, Weibull, Gamma Multi-hit models.
** For comparison.

Table 10-7. Males with Tumors -- Additive Background Models

Exposure LADE mg/kg/day	Model*	MLE	UB or UCL
8.2×10^{-3}	L.M.**	9×10^{-3}	1×10^{-2}
	A.P.	9×10^{-2}	3×10^{-1}
	A.L.	1×10^{-1}	4×10^{-1}
	A.W.	1×10^{-1}	4×10^{-1}
	A.G.	9×10^{-2}	3×10^{-1}
1.4×10^{-5}	L.M.**	2×10^{-5}	2×10^{-5}
	A.P.	2×10^{-4}	8×10^{-4}
	A.L.	2×10^{-4}	9×10^{-4}
	A.W.	2×10^{-4}	1×10^{-3}
	A.G.	2×10^{-4}	8×10^{-4}

* Linearized Multistage, and Additive Background Probit, Logit, Weibull, Gamma Multi-hit Models.
** For comparison.

Table 10-8. Females with Tumors --Additive Background Model

Exposure LADE mg/kg/day	Model*	MLE	UB or UCL
8.2×10^{-3}	L.M.**	3×10^{-2}	3×10^{-2}
	A.P.	6×10^{-2}	1×10^{-1}
	A.L.	6×10^{-2}	1×10^{-1}
	A.W.	5×10^{-2}	1×10^{-1}
	A.G.	7×10^{-2}	2×10^{-1}
1.4×10^{-5}	L.M.**	6×10^{-5}	4×10^{-5}
	A.P.	1×10^{-4}	2×10^{-4}
	A.L.	1×10^{-4}	2×10^{-4}
	A.W.	9×10^{-5}	2×10^{-4}
	A.G.	1×10^{-4}	3×10^{-4}

* Linearized Multistage, and Additive Background Probit, Logit, Weibull, Gamma Multi-hit Models.
** For comparison.

Table 10-9. Females with Tumors -- Independent Background Models

Exposure LADE mg/kg/day	Model*	MLE	UB or UCL
8.2×10^{-3}	L.M.**	3×10^{-2}	3×10^{-2}
	I.P.	3×10^{-4}	2×10^{-3}
	I.L.	7×10^{-3}	3×10^{-2}
	I.W.	2×10^{-2}	7×10^{-2}
	I.G.	1×10^{-2}	5×10^{-2}
1.4×10^{-5}	L.M.**	6×10^{-5}	4×10^{-5}
	I.P.	- 0 -	- 0 -
	I.L.	3×10^{-8}	3×10^{-7}
	I.W.	9×10^{-7}	9×10^{-6}
	I.G.	6×10^{-8}	5×10^{-7}

* Linearized Multistage, and Independent Background Probit, Logit, Weibull, Gamma Multi-hit Models.
** For comparison.

Table 10-10. Females with Malignant Tumors -- Additive Background Models

Exposure LADE mg/kg/day	Model*	MLE	UB or UCL
8.2×10^{-3}	L.M.**	8×10^{-3}	1×10^{-2}
	A.P.	2×10^{-2}	6×10^{-2}
	A.L.	2×10^{-2}	5×10^{-2}
	A.W.	2×10^{-2}	5×10^{-2}
	A.G.	2×10^{-2}	6×10^{-2}
1.4×10^{-5}	L.M.**	9×10^{-6}	2×10^{-5}
	A.P.	3×10^{-5}	9×10^{-5}
	A.L.	3×10^{-5}	9×10^{-5}
	A.W.	3×10^{-5}	9×10^{-5}
	A.G.	3×10^{-5}	1×10^{-4}

* Linearized Multistage, and Additive Background Probit, Logit, Weibull, Gamma Multi-hit Models.
** For comparison.

with the linearized multistage procedure (section 10.2.) were used here. Of the eight models (independent and additive background for each model), male rats with tumors-independent background and female rats with malignant tumors - independent background, failed to produce estimates because of the fit of the data (basically how the model incorporated the background response observed in the study). Generally, the risk estimates are about as high or higher than those from the linearized multistage procedure. Only the probit-independent background, females with tumors, produced very low risks.

10.3. Uncertainty Due to Exposure Estimation

Often the exposure analysis of a risk assessment is weak and it is, therefore, capable of introducing a high level of uncertainty into the risk estimation process. In the case of acrylamide, however, the exposure assessment contains recent, validated data. The NIOSH study produced data for the three domestic manufacturers of acrylamide as well as for a significant number of the processing sites. The confidence in these data are increased because of the nearly identical manufacturing and processing procedures currently in use. In addition, the number of workers at these facilities is reliably known.

The grouting segment, while not as widely surveyed as the manufacturing and processing segments, has been well characterized. A combination of surveillance and monitoring site visits has provided a clear understanding of work practices and

quantitative estimates of exposure. As with the manufacturing/processing segments, the exposed population is reasonably well-known.

The laboratory population using polyacrylamide gels is relatively large, 100,000 to 200,000 persons, but exposure may be minimal because of the use and handling characteristics of this segment. Only the downstream populations are not as well-characterized. However, in these instances, exposure to acrylamide results from residual levels in polyacrylamide products such as water treatment chemicals. For instance, while industry-supplied data have bounded acrylamide concentrations in drinking water (at least in the treatment plant), the duration of exposure and population size are not as well-known. A similar situation exists for sugar consumption.

In summary, the manufacturing/processing and grouting segments are well-characterized regarding numbers of persons exposed, duration of exposure, and exposure levels. These groups also experience the highest cancer and noncancer risks. In contrast, those groups exposed to contaminant/residual levels of acrylamide are not as well-characterized, but risks should logically be much lower.

10.4. Summary

As Tables 10-1, 10-2, and 10-4 indicate, persons involved in sewer grouting face potential cancer risks of 10^{-3} to 10^{-1} as well as margins of exposure (MOE) for neurotoxic and reproductive

Finally, although the risk of heritable mutations is difficult to quantify, the qualitative weight of evidence is high. Only human evidence could make the case stronger. Consequently, in the absence of information to the contrary, all exposed groups must be considered to be at some level of risk, with grouting workers at the highest level followed by manufacturing/processing employees.

11. RISK CHARACTERIZATION

This risk characterization presents the major conclusions of EPA's risk assessment of acrylamide. Acrylamide is a known neurotoxin. Animal studies have established effect levels and have begun to elucidate its mode of action in causing neurotoxicity. Other studies show that acrylamide can cause reproductive and developmental effects in laboratory animals. Subchronic and chronic exposure studies, corroborated by other scientific evidence, support a conclusion that exposure to acrylamide poses a risk of cancer for humans. Also, the data indicate a strong potential for heritable mutations in the human population.

The risk characterization reviews the underlying scientific foundation for these findings and describes the strengths and weaknesses of supporting data. It is divided into three sections which discuss the qualitative aspects of the risk assessment, the exposure, and the quantitative risk estimations at current exposure levels.

11.1. Hazard

The available data provide a basis for identifying several human health hazards related to acrylamide exposure, but with varying degrees of confidence. In order of decreasing confidence, the identified hazards include: neurotoxicity, carcinogenicity, genotoxicity (heritable mutations) reproductive

effects, and developmental effects. There is a strong case for identifying neurotoxicity, carcinogenicity, and heritable mutations, a moderate case for identifying reproductive effects, and a weak case for identifying developmental effects of acrylamide exposure. The available evidence of these hazards is sufficient to conduct quantitative risk assessments for neurotoxicity, carcinogenicity, and reproductive effects, to a limited extent for heritable mutations, but is not sufficient for such purposes for developmental effects.

The strongest data set is that which identifies neurotoxicity from acrylamide exposure. This effect has been observed in both humans and laboratory animals. The evidence identifying this effect is incontrovertible based on observations of neurotoxic effects in both the peripheral and central nervous systems of humans, by the irreversibility of effects in some human case reports, by the cumulative effects of chronic exposures, and by the presence of a dose-response (from animal studies). The best animal data indicate NOELs and LOELs of 15 and 25 mg/kg, respectively, following a single dose, and 0.2 and 1.0 mg/kg/day, respectively, following chronic exposures. Also, in the animals tested there does not seem to be any appreciable difference in effect levels. In rats, cats, and monkeys the LOEL is 1.0 mg/kg/day.

Exposure to acrylamide by a variety of routes may produce serious neurological effects in humans. The human reports of neurotoxicity also indicate that although most of the individuals

recovered completely after the exposure was stopped, some severely affected persons did not appear to recover completely following cessation of the exposure, indicating an irreversible effect.

Acrylamide destroys the most distal axons of both central and peripheral neurons and interferes with retrograde axonal transport. It also produces a number of biochemical effects that may or may not be relevant to its neurotoxicity. The precise mechanism of action by which acrylamide produces neurotoxicity is unknown.

There are 53 human cases of acrylamide toxicity reported in the literature, all but 5 related to occupational exposure. In these occupational cases dermal exposure predominates. As is typical in such cases, the exposure levels are only qualitatively known. However, the severity of effects in these reports does seem to be a function of exposure duration.

A recent field monitoring study sponsored by EPA at sewer grouting sites found one case of neurotoxicity and documented two reports of previous neurotoxic symptoms. As opposed to the cases mentioned above, inhalation and dermal exposures were measured at the grouting sites. The monitoring indicated that significant dermal exposure and risks can be expected. (See section 11.3.)

The early signs and symptoms of exposure noted in these cases include: skin peeling and other skin changes, numbness and tingling of the extremities, coldness of skin, excessive sweating, bluish-red skin color, and muscle weakness. These are

generally followed by fatigue, confusion and other psychological effects, gastrointestinal problems, and weight loss. These signs typically precede the dramatic findings of overt peripheral nerve dysfunction (i.e., ataxia and weak or absent tendon reflexes). These may be followed by an inability to stand, body tremors, slurred speech, difficulty in swallowing and other signs. In general, victims showed complete recovery, although not always. Individuals with relatively mild symptoms recovered completely following cessation of exposure. In summary, the human case reports present evidence of both central and peripheral nervous system effects following short- and long-term exposures to acrylamide. In principle, central effects are much more likely to be irreversible if neural damage takes place and are, therefore, of greater concern.

In only one human case study can exposure be estimated. A Japanese family of five individuals was exposed through ingestion and external use of well water contaminated with acrylamide, and the exposure was estimated to be about 11.4 mg/kg as a daily dose and lasted about one month when symptoms developed. The symptoms and doses correspond fairly well to data from prolonged exposure in cats and monkeys. Even so, the human data are quantitatively inadequate for risk assessment because few data on human exposure levels exist. Based on recent and older studies of prolonged exposure in rats, cats, and monkeys it appears that prolonged exposure to 1 mg/kg/day or more causes neurotoxic effects.

Based on the available data there is evidence according to EPA's Cancer Risk Assessment Guidelines to identify acrylamide as a "B2-probable human carcinogen." The evidence for this effect of acrylamide is relatively strong and is strengthened by the following: occurrence of benign and malignant tumors, tumors observed in males and females, tumors observed at multiple sites, tumors observed in 2 animal species (one tested in a lifetime bioassay at very low doses and one tested in limited bioassays), and a dose-response observed in a lifetime bioassay. A final report on the 2nd lifetime Oncogenicity Study in Rats with Acrylamide was completed on June 27, 1989 (American Cyanamid Company, Final Report, June 27, 1989). This study confirmed the multiple-sites carcinogenicity of acrylamide in the rat. The prior carcinogenicity study conducted by Johnson et al. (Toxicol. Appl. Pharmacol. 85:154-168, 1984) showed similar indications, although results of the two studies differed somewhat regarding specific incidences. This constitutes "sufficient" evidence of carcinogenicity from animal studies. Also, the genotoxicity data indicate that acrylamide can interact with and damage DNA material. Three epidemiologic studies have been reviewed. One study showed a slight increase in lung and central nervous system cancers. However, the study cannot be characterized as an adequate appraisal of potential cancer risk because of the need for more complete information concerning workers' exposure and mortality experience. The other study, while not showing any increase in malignancies in a workplace cohort when workers with

previous dye exposure were excluded, suffered from small cohort size, limited follow-up and low exposure (in years). The third study was ambiguous in its definitions of the study groups as well as having inadequate exposure data concerning acrylamide and no data concerning other exposures. Consequently, the available human data are "inadequate" under the Guidelines to judge a human cancer hazard from acrylamide.

Although the lifetime bioassay in rats was a drinking water study and some populations are exposed to acrylamide by the dermal and inhalation routes, the available information on the pharmacokinetics of acrylamide indicates that the applied dose whether by the oral, dermal, or inhalation routes is probably directly proportional to internal effective dose over a range of dosing conditions.

Acrylamide has none of the properties that suggest major non-linearities between applied dose and internal dose. It is rapidly and completely absorbed by the gut. It is quite uniformly distributed among tissues and, being quite polar, does not tend to accumulate in adipose tissue. It is rapidly cleared by metabolism. The proportionality of tissue accumulation of radioactive labeled acrylamide to applied dose holds over a range of tested dosing rates and multiple dosing patterns do not introduce any non-proportionalities. Thus there does not appear to be the potential for major differences in the carcinogenic potential of acrylamide by route of exposure.

The genotoxicity data on acrylamide provide a basis to support the carcinogenicity hazard identification and to support mutagenicity as a separate endpoint of concern for acrylamide exposures. The major concern for the genotoxicity of acrylamide is its clastogenic activity, which appears more pronounced in the germ cells than in somatic cells. The interaction with the germinal tissue suggests the possible heritability of acrylamide induced mutations.

The evidence identifying this effect is strengthened by the consistent pattern of the laboratory results and by the strong results in a mouse heritable translocation assay.

The EPA guidelines for mutagenicity risk assessment allow for an evaluation of the potential genetic risk associated with human exposure to acrylamide. The genotoxicity data for acrylamide suggest an intrinsic mutagenic (clastogenic) activity and provide sufficient evidence for chemical interaction in the mammalian gonad. The body of evidence suggests that acrylamide may induce alterations in the genome the germinal cells. These data provide a fairly strong weight of evidence bearing on the potential for human germ-cell mutagenicity and its heritability. The results of a heritable translocation assay increase the strength of a human germ-cell mutagenicity concern to a level that would only be further strengthened by direct human evidence (the highest level of evidence for human mutagenicity).

Additional data (at multiple doses) are necessary from the mouse heritable translocation assay to establish a dose-response and to strengthen quantitative risk estimation of this endpoint.

A reproductive hazard from acrylamide exposure has been identified based on the results of several animal studies. Reproductive effects of acrylamide were observed both physiologically and biochemically. The doses at which these reproductive effects were observed also produced other toxic effects in all the animals tested, except in a few instances. Consequently, the available data indicate that acrylamide can act directly on the reproductive system, rather than indirectly through stress or other systemic effects such as neurotoxicity. Thus the concern for the reproductive toxicity potential is heightened.

The LOEL and NOEL for reproductive effects, as indicated by the available data (Table 6-2 in section 6-1.), are 8.8 and 4.2 mg/kg/day, respectively.

Although there is some evidence that acrylamide may cause developmental effects, many of the available reports indicate that developmental effects occur only in the presence of maternal toxicity. However, two reports, one involving biochemical changes of unknown significance and another involving neurotoxicity in the pups do provide some limited basis for identifying a developmental hazard from acrylamide exposure. Unless further study elucidates the developmental hazard, concern

for other effects should be emphasized over these developmental effects.

While the available data provide a good general understanding of the metabolism of acrylamide (including: absorption, distribution, biotransformation, and elimination), there is still a lack of detailed pharmacokinetics for certain aspects of its metabolism. For example, there is currently a lack of specific dermal absorption rates under various conditions, although the data are adequate to place bounds on these rates. However, recent studies of the mechanism of action of acrylamide's toxicity, especially its neurotoxicity, have begun to yield an understanding of some the processes or mechanisms through which acrylamide produces its various toxic effects.

11.2. Exposure

The body of exposure data is relatively extensive for the manufacturing/processing and sewer grouting sectors, less so for the others. Monitoring was performed by NIOSH at the three domestic acrylamide manufacturing sites. These sites also include facilities to convert acrylamide to various polyacrylamide products. The NIOSH survey produced validated exposure data that is in good agreement with concurrently produced data by one of the companies monitored. The one drawback of the NIOSH survey is that dermal exposure was only identified as a potentiality based on equipment wipe samples. Even so, an analysis of the production process and a review of

the safety equipment routinely used indicates a low potential for dermal exposure.

While not as extensively monitored as manufacture/ processing, the sewer grouting sector is well-characterized regarding both inhalation and dermal exposure. Because the grouting procedure and equipment used are relatively uniform throughout the country (there are only two major suppliers of grouting equipment), the data developed from the geographically limited site surveys could be applied country-wide. The NIOSH data for a sewer grouting site agree well with the sites monitored by EPA. An uncertainty regarding the dermal exposure estimates is the rate of absorption. It was assumed that up to 25 percent of the recovered acrylamide would be absorbed. This is not an overly conservative estimate and it agrees well with the animal data.

The two major consumer exposures, drinking water and sugar consumption, can only be estimated because of a lack of monitoring data. (Polyacrylamide products, which contain residual acrylamide monomer, are used as flocculants to clarify raw water in potable water plants and as clarifying agents in sugar refining.) However, based on maximum allowable and typical usage rates for the polyacrylamide products used, a range of exposures can be presented with some confidence.

No estimates can be provided for the other exposure categories, these mostly include users of polyacrylamide products which contain residual acrylamide and were not a focus of the

assessment. Direct users of acrylamide such as by laboratory personnel also have some potential for acrylamide exposure, but this could vary greatly from laboratory to laboratory.

11.3. Risk Estimates

Three endpoints were quantified, cancer, neurotoxicity and reproductive effects. The risk of heritable mutations was examined, but specific estimates of risk are not recommended. Since adequate human data were not available, estimates of cancer risk were generated using animal data from a long-term bioassay. Statistically significant tumor incidence data in animals were pooled for purposes of quantitative risk estimation, since risk numbers derived from site-specific tumor incidence data may not be predictive of (and may in fact underestimate) "whole-body" risks that are determined using the pooled individual animal data. The level of risk for neurotoxicity and reproductive effects was determined by calculating margins of exposure.

Since risks at low exposure levels cannot generally be measured directly either by experiments in animals or by epidemiologic studies, a number of mathematical models have been developed to extrapolate from high to low doses. The Office of Science and Technology Policy published principles on model selection which states that "No single mathematical procedure is recognized as the most appropriate for low-dose extrapolation in carcinogenesis. When relevant biological evidence on mechanism of action exists, the models or procedures employed should be

consistent with the evidence. When data and information are limited, however, and when much uncertainty exists regarding the mechanism of carcinogenic action, models or procedures which incorporate low-dose linearity are preferred when compatible with the limited information." Data relevant to selecting a model for cancer risk extrapolation associated with exposure to acrylamide were reviewed.

As a result of that review, the linearized multistage model was used to estimate cancer risks. An analysis of the data base did not indicate that another model would be more appropriate or that the doses should be adjusted to reflect different patterns of distribution or metabolism. The distribution of acrylamide appears to be quantitatively the same regardless of route of exposure. Consequently, as indicated by EPA's Cancer Assessment Guidelines and in the absence of information to the contrary, the linearized multistage model was used.

Estimated excess lifetime individual cancer risks for various exposure categories are presented in Table 11-1. The highest upper-bound risks are for sewer grouting workers, with risks ranging from 10^{-2} to 10^{-1}. Risks for manufacturing personnel range from 10^{-3} to 10^{-2} based on recent NIOSH monitoring data. Consumers of drinking water face risks of about 10^{-6}. Other exposed groups face unknown levels of risk due to a lack of exposure information. However, except for lab workers, the other groups would be exposed to acrylamide as a contaminant

(or residue) in polyacrylamide products where the exposure potential is expected to be very low.

Table 11-1. Summary of Estimates of Upper-Bound Individual Excess Lifetime Cancer Risks Associated with Exposure to Acrylamide

Exposure Category	Upper-Bound Individual Risk Estimates
Soil Grouting	10^{-2} - 10^{-1}
Manufacturing/Processing	10^{-3} - 10^{-2}
Drinking Water	10^{-6} - 10^{-5}*

* Worst-case to typical exposure based on residual acrylamide allowed.

Other models were also used to estimate risk, such as the independent and additive background logic, probit, Weibell, and gamma multi-hit models. In general, similar or higher risks were obtained with these models as compared to the linearized multistage model.

The available hazard data for both reproductive effects and neurotoxicity are adequate for risk estimation because well-conducted studies are available to identify NOEL's and LOEL's. The data are not sufficient to do this for developmental hazards.

As a way to measure this risk, margins of exposure (MOE) were calculated using the NIOSH, EPA, and industry supplied data. For neurotoxicity a NOEL of 0.2 mg/kg/day was used for risk estimation. A reference dose (RfD) of 0.0002 mg/kg/day was developed using this NOEL and an uncertainty factor of 1000 (UF).

The NOEL and RfD will be used to evaluate neurotoxic risks. A NOEL of 4.2 mg/kg/day was used for reproductive risks. An MOE is simply a measure of the proximity of an environmental exposure to a NOEL or LOEL determined in a laboratory study (NOEL divided by human exposure level). For instance, if a NOEL of 0.3 mg/kg/day were used and the exposure was 0.1 mg/kg/day, then the MOE would be 3. The lower the MOE the higher the concern. Alternatively, estimated human exposure can be compared to the RfD either directly or whether the MOE is less or greater than the UF (X any modifying factor if appropriate) used to determine the RfD. (See section 10.1. for a discussion of RfD's.)

The NIOSH and EPA monitoring data indicate a high potential for neurotoxicity in some groups. As Table 11-2 and Figure 11-1 indicate, MOE's for sewer grouting are less than 10 and are significantly lower than the UF of 1000 used to set the oral RfD of 0.0002 mg/kg/day. When viewed on a dose basis (Figure 11-1(b)) two sites exceeded the OSHA standard. This supports the finding that one of the nine employees observed during EPA's field monitoring survey exhibited signs of acrylamide induced neurotoxicity and two others reported past episodes of neurotoxicity, which indicates that many grouting workers may be exposed to levels of acrylamide close to the estimated human effect level (about 1 mg/kg). In the manufacturing/processing sector, MOE'S ranged from 23 to 282. No signs of neurotoxicity were observed at any of the sites surveyed.

In studies where both reproductive and neurotoxic effects have been seen, the neurotoxic effects were seen at lower dose levels. The evidence of neurotoxic effects is unquestionable; both animal effects have been observed. Adequate data are available to show that there are no major differences in effect levels in animals and limited evidence that similar levels cause neurotoxic effects in humans. In contrast, the weight of evidence for reproductive effects in animals is not as high as for neurotoxicity. The MOE's for reproductive effects are higher than those for neurotoxicity. Reproductive effects have been seen in some studies in the absence of neurotoxic effects but not in others. The risk presented for reproductive effects is not considered as high as for neurotoxicity at a given exposure level.

Consumers of water have MOE's greater than 1000. Laboratory personnel may be at risk, but the level of risk is difficult to gauge without better exposure data. Persons who use polyacrylamide products are expected to have high MOE's based on the low exposure potential.

Finally, acrylamide appears to be a potent clastogenic agent in germ cells. This presents the possibility for the heritability of acrylamide induced mutations. Under EPA's mutagenicity guidelines only human evidence could make the case stronger. Consequently, in the absence of information to the contrary, all exposed groups must be considered to be at some

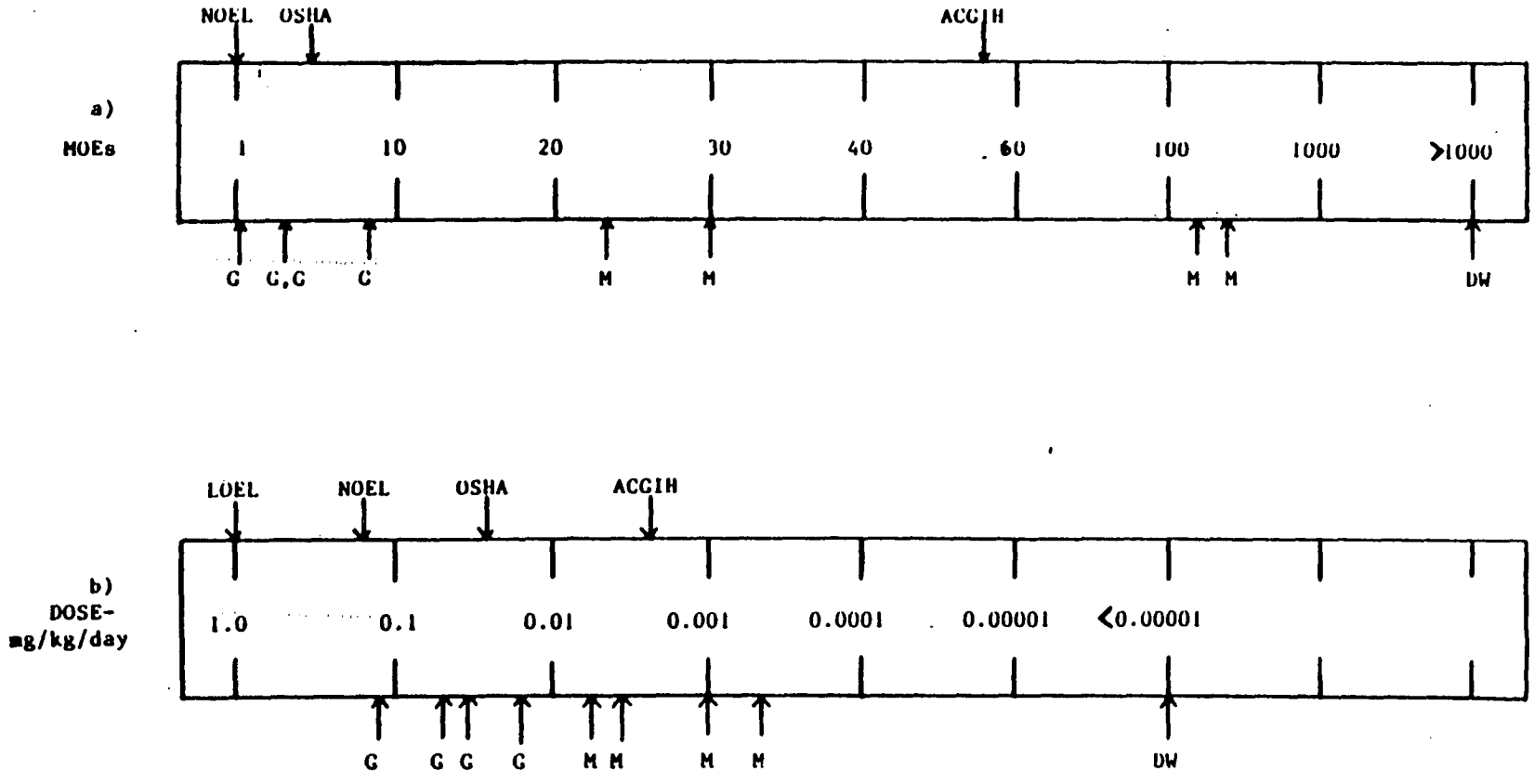


Figure 11-1. Summary of neurotoxicity risks, exposures, and exposure standards. G=Grouting site. M=Manufacturing/Processing site. DW=Drinking water.

Table 11-2. Summary of Noncancer Risks Expressed as Margins of Exposure Associated with Acrylamide Exposure

A. Neurotoxicity

Range of MOE's	Exposure Groups
≤10	OSHA Standard, Grouting Sites (EPA)
11-50	Cyanamid, Dow (M)
51-100	
101-1000	NALCO, Dow (P), Soil Grouting (NIOSH)
> 1000	Drinking Water

B. Reproductive Effects

Range of MOE's	Exposure Groups
20-200	OSHA Standard, Grouting Sites (EPA)
210-700	Cyanamid, Dow (M)
700-4000	
>4000	Dow (P), NALCO, Soil Grouting (NIOSH)
$>10^5$	Drinking Water

level of risk. A limited quantitative analysis indicates that certain occupational settings (i.e., soil grouting) may be associated with dominant lethal effects at risk levels about two orders of magnitude below estimated lifetime excess cancer risks. The corresponding risk for heritable translocations may be as high as the dominant lethal risks but may be orders of magnitude lower than that. Even though these risk estimates for heritable effects are only preliminary, they should not be totally ignored. They should be considered along with risks associated with acrylamide's ability to produce neurotoxic and carcinogenic effects. The risk from acrylamide exposure can then be conceived as being a function of its ability to produce several different adverse effects and not just a single endpoint. In order to portray the heritable risks more reliably, further dominant

lethal and heritable translocation test results would be required, hopefully using the same multiple-dose levels.

12. SUMMARY OF PUBLIC COMMENTS AND EPA RESPONSES ON PRELIMINARY ASSESSMENT OF HEALTH RISKS FROM EXPOSURE TO ACRYLAMIDE

Comments were submitted by the four following concerned public groups: Synthetic Organic Chemical Manufacturers Association, Inc., Nalco Chemical Company, the Dow Chemical Company, and the American Cyanamid Company. The corresponding responses were submitted by the U.S. Environmental Protection Agency.
The comments (C) and responses (R) are categorized into six major areas of interest: carcinogenesis, genotoxicity, neurotoxicity, reproductive effects, exposure, and risk assessment.

12.1. Carcinogenesis: (C) The 2-year chronic bioassay performed by the Acrylamide Producers Association does not provide adequate support to classify acrylamide as a B2 carcinogen. Results were distorted by including data on benign tumors in the total pool of tumors. A corrected Table 10.3 has been submitted. A second carcinogenicity study completed by the American Cyanamid Company is being analyzed.

While an increased cancer incidence occurred when a promoter treatment followed acrylamide, the promoter also showed tumorigenic activity.

Two human epidemiology studies showed acrylamide is not carcinogenic to humans at levels of worker exposure.

(R) The B2 classification is supported by several studies. Using some of the same data, acrylamide has been classified as a

Group 2B carcinogen by the International Agency for Research of Cancer.

The results from the second carcinogenic study will not negate the positive responses found in the first study.

Risk assessment should not be based upon initiation activity if no promoter is known to be present. The risk of acrylamide may be greater in mixtures than alone.

The human studies performed are inadequate to correlate exposure and incidence.

12.2. Genotoxicity: (C) Genotoxic effects occur when acrylamide interacts with cytoskeletal proteins such as microtubules and microfilaments. This protein interaction may explain why chromosomal effects can be produced without point mutations.

(R) Acrylamide can alkylate DNA in vitro, induce aberrations, and bind to testicular DNA in vivo.

12.3. Neurotoxicity: (C) Using electron microscopy, chronic tests determined the lifetime neurotoxic NOEL to be approximately 0.5 mg/kg/day. The 90-day NOEL of 0.2 mg/kg/day is derived only from a range-finding study. The better data using a 0.5 mg/kg/day has been ignored.

Neurotoxicity occurs at the same dose in the reproductive studies as in the neurotoxicity studies. The measure of neurotoxicity many times is the gross observation of weakness of

the hind limbs. No histology was performed nor were quantitative evaluations of hind limb alterations conducted. The cat study of acrylamide-induced hind limb weakness is not acceptable as all control animals in the study died.

The study on acrylamide-induced alterations of retrograde transport to determine the single-dose NOEL and LOEL is questionable since the functional deficit was not evaluated.

No evidence of neurotoxicity was identified in a screening test of workers associated with the current standard concentration of 0.3 mg/m3. No trend toward elevated mean scores from the Optacon Tactile Tester have been observed either.

Data exists stating that acrylamide and its neurotoxic analogs reduced colchicine-binding in the sciatic nerve, but not in the brain and cerebellum.

(R) The NOEL OF 0.2 mg/kg/day was accepted because electron microscopy, the more sensitive method for endpoint measurement, was used. Light microscopy was used to determine the 0.5 mg/kg/day dosage. EPA is required to be conservative in setting reference doses and making risk estimates.

12.4. Reproductive and Developmental Toxicity: (C) Calculations for the NOEL and LOEL were not based upon a mg/kg/day dosage, but rather upon a mg/animal/day dosage. Corrected dose levels are submitted. The method used for detection was not sensitive enough to determine neurotoxicity at lower concentrations.

A NALCO study showed no tibial nerve changes when pups were exposed to acrylamide throughout gestation and lactation, but instead showed tibial nerve lesions, weight gain, and possible clinical effects at different dosages.

(R) The following revised NOELs AND LOELs are essentially the same as those offered in the comments and should be included in the corrected document:

female reproductive effects:	LOEL = 17.4 mg/kg/day noted, NOEL = 13 mg/kg/day
male reproductive effects:	LOEL = 8.8 mg/kg/day NOEL = 4 mg/kg/day

The study was done in mice, not rats.

The findings of developmental and reproductive toxicity would not be altered by coincident neurotoxicity.

The results of the Nalco study do not eliminate the concerns for developmental toxicity.

12.5. Exposure: (C) The total domestic involvement in acrylamide manufacture is less than 100. It is estimated that there are 40 polymer plants in this country with fewer than 5 people unloading acrylamide. Protective clothing and equipment is widely used by workers.

The average years-of-exposure for acrylamide manufacturing/ processing was determined to be 6.5 years. No adjustment for less-than-lifetime exposure was made.

(R) The total number of workers exposed at the four manufacturing sites and the 40 polymer processing sites should range from 500 to 1,000.

Uncertainties in the manner and frequency of using protective equipment prevents us from considering their use in exposure assessments. The estimate of 10,000 workers potentially exposed to acrylamide in 27 occupations was based on the National Occupational Hazard Survey conducted by NIOSH.

Potential exposure to acrylamide from polyacrylamide gel electrophoresis has not yet been determined.

12.6. Risk Assessment: (C) The linearized multistage model does not fit the purported cytoskeletal protein binding mechanism of action of acrylamide for data in the observable range.

Regarding the factor of 5.85 for interspecies extrapolation, toxicological effects from acrylamide occur in the same dose range regardless of species. A metabolic rate extrapolation between species based on a surface area correction is inappropriate. Dose extrapolation is better done on a mg/kg basis which would reduce the risk by a factor of 5 to 10.

The 2-year study provides a NOEL estimate of 0.5 mg/kg/day derived from exposure as long as 12 months. Maximum Observed Effects (MOE) should be included in the document without including company names.

The requirement for multiple chromosomal modifications to effect DNA damage indicates that a threshold model would better represent the data. A multiple hit model should be invoked.

Since the human epidemiology study showed no increased incidence of cancer, the risk calculated by the EPA is inaccurate.

Calculations for exposure and the margin of exposure for grouting sites in Table 10.1 should be corrected.

(R) The use of nonthreshold models is appropriate when low-dose linear behavior cannot be ruled out. The multistage model does fit the data in the observable range.

An interspecies extrapolation factor of 7.05 was used for risk assessment. The acrylamide risk estimation procedure follows that of the EPA Guidelines for Mutagenicity Risk Assessment.

NOELs across species range from 0.2 to 2.0 mg/kg. LOELs are very similar across species at 0.1 mg/kg. After calculations are made, the range of reference dose for preventing chronic neurotoxic effects is 1-9 ug/kg/dy.

All that is required for linearized multistage models such as those used here are multiple biological events. The number of chromosome modifications needed to induce enough strain to result in DNA breakage could depend on the location affected.

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